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VOLUME 43 DECEMBER, 1953 NUMBER 12

PART I

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Badger Market, a new disease resistant cabbage
J. C. Walker, R. H. Larson, and G. S. Pound 649

Measurement of resistance in alfalfa to common leaf spot — Fred Reuel Jones 651

Factors affecting the partial vacuum inoculation of seedling corn with Ustialgo zeae
J. B. Rowell and J. E. DeVay 654

Some chemotherapeutic agents for wheat stem rust — Hugh Howison Hotson 659

The cause of epinastic symptoms in Fusarium wilt of tomatoes
A. E. Dimond and Paul E. Waggoner 663

The relation of host nutrition to multiplication of turnip virus 1 in Nicotiana glutinosa and N. multivalvis — Glenn S. Pound and Lewis G. Weathers 669

Heat of respiration of injured and diseased leaves — C. E. Yarwood 675

Further studies on physiologic races of Ustilago tritici — Wayne M. Bever 681

Identity of potato curly top and green dwarf — J. D. Menzies and N. J. Giddings 684

Further studies on the electron microscopy of purified tobacco ringspot virus
P. R. Desjardins, C. A. Senseney, and G. E. Hess 687

Transmission of phony disease virus from tops of certain affected peach and plum trees
Lee M. Hutchins, L. C. Cochran, W. F. Turner, and J. H. Weinberger 691

An improved method of inoculating seed of oats and barley with smut
W. Popp and W. J. Cherewick 697

Phytopathological Notes:

Sterile conks of Fomes igniarius on aspen — S. B. Silverborg 699

An improved method of isolating Fusarium spp. from plant tissue
R. H. Stover and B. H. Waite 700

A mechanism for the control of common rootrot of wheat
S. H. F. Chinn, R. J. Ledingham, B. J. Sallans, and P. M. Simmonds 701

Oospore germination in Peronospora tabacina — L. H. Person and G. B. Lucas 701

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. . . *THROUGH CHEMISTRY*

BADGER MARKET, A NEW DISEASE RESISTANT CABBAGE [1]

J. C. Walker, R. H. Larson, and G. S. Pound

It has been shown in previous papers that resistance to yellows incited by *Fusarium oxysporum* f. *conglutinans* (Wr.) Snyder & Hansen in cabbage (*Brassica oleracea capitata* L.) is controlled by a single gene pair[2] and that resistance to mosaic incited by turnip virus 1 and cauliflower virus 1 is polygenic.[3] The breeding program which has led to the development of Badger Market has had 3 major objectives: 1) to secure a second early, round head variety which produces a small, compact head suited to current fresh market requirements; 2) to maintain yellows resistance; 3) to maintain, and if possible improve, the degree of mosaic resistance now available in most early and second early varieties. Two yellows-resistant varieties, Racine Market and Globe, which have already been described,[4] were used as parental stocks. After individuals from each variety were crossed several successive self generations were grown and selections for type and mosaic resistance were made. From a series of F_5 progenies the one with the most suitable combination of type and resistance was chosen. This progeny was increased by sib-mating of selected individuals in 2 successive generations. Each mass increase was subjected to extensive commercial trials. The second mass increase was released to primary seed growers in April 1952 as the foundation stock of a new variety called Badger Market. The United States Department of Agriculture and the Wisconsin Agricultural Experiment Station announced in October, 1953, that seed was available for general use. The purpose of this paper is to record the principal type and disease resistant characters of Badger Market.

FIG. 1. Plant and head type of Badger Market.

DESCRIPTION OF BADGER MARKET.—The terms used for description of the variety follow the standards used by Boswell et al.[5] Outermost leaves are horizontal to slightly upright; inner leaves are at about 45° above horizontal. Leaf tops are rounded; leaf bases are spatulate to broadly spatulate. The leaf surface is smooth and slightly concave to concave. Leaf margins are smooth; leaf edge is crenate. Leaf veins and ribs are slightly prominent. Color of leaf is bluish green coinciding closely with that of Racine Market. Heads are prominent, nearly spherical, well rounded at top and base. Outer head leaves are tightly clasping, with moderately prominent ribs. Inner head leaves are crumpled, giving a very compact internal structure. Plant type and head type are shown in Fig. 1.

PHYSICAL MEASUREMENTS.—In Table 1 are certain of the physical characters of Badger Market in comparison with those of a number of standard varieties including the parents of the new introduction. These data were secured from plants started in cold frames and transplanted out of doors at Madison, Wisconsin, on June 5, 1950. Badger Market matures about a week later than the standard early round variety Golden Acre and somewhat earlier than either parent. The smaller size of head obtained in this trial was maintained consistently in other tests. The polar-equatorial diameter ratio shows the average head to be spherical, as is the case with both parents and of the other varieties used in the comparison. The ratio of polar diameter to core length is close to that of Globe but the new

[1] Accepted for publication May 12, 1953.

[2] Walker, J. C. 1930. Inheritance of *Fusarium* resistance in cabbage. Jour. Agr. Res. [U. S.] 40: 721-745.

[3] Pound, G. S., and J. C. Walker. 1951. Mosaic resistance in cabbage. Phytopathology 41: 1083-1090.

[4] Walker, J. C., and J. P. Jolivette. 1948. Yellows-resistant varieties of cabbage in the early and midseason round-head groups. U. S. Dept. Agr. Cir. 775.

TABLE 1.—*Physical measurements of Badger Market in comparison with certain other standard varieties, Madison, Wis., 1950*

Variety	Days from transplanting to harvest	Ave. no. of outer leaves	Ave. wt. of outer leaves (gm.)	Ave. wt. of head (gm.)	Ave. equatorial diameter of head (cm.)	Ave. polar diameter of head (cm.)	Ave. core length (cm.)	Ratio of polar to equatorial diameter	Ratio of polar diameter to core length	Ratio of head weight to leaf weight
Golden Acre	57	14.3	1072	2594	19.6	19.2	12.1	0.98	1.59	2.42
Resistant Detroit	64	14.3	1021	3004	20.8	19.9	12.3	0.96	1.62	2.94
Badger Market	64	13.6	944	1958	17.6	17.8	8.8	1.01	2.02	2.07
Wisconsin Copenhagen	70	13.2	1525	3275	21.5	20.9	11.5	0.97	1.82	2.15
Racine Market	73	12.8	1085	3337	21.4	21.4	15.0	1.00	1.43	3.08
Marion Market	77	15.2	1651	3351	21.2	20.5	11.2	0.97	1.83	2.03
Globe	86	17.5	2152	3803	22.1	21.5	10.5	0.97	2.05	1.77
LSD 19:1		1.1	144	347	1.0	0.6	0.8			
99:1		1.5	195	468	1.3	0.8	1.0			

variety is distinct in this regard from Racine Market, which has an objectionably long core.[5] The ratio of head to leaf weight is intermediate between the ratios of the 2 parents.

DISEASE RESISTANCE.—Badger Market was shown by numerous tests of seedlings in infested soil at 24° C. to be homozygous for single-gene resistance to yellows. Both parent varieties are moderately susceptible to mosaic but less susceptible than most other varieties in the early and midseason round groups. In the breeding program, progenies were inoculated with turnip virus 1 and cauliflower virus 1 according to methods already described.[3] Selections were made, where desired type plants were available, within the most tolerant progenies. In 1952 Badger Market was compared by the same methods with the parent-varieties and with several other yellows-resistant varieties within the early and midseason round head groups. A disease index was calculated for each variety, the possible range of index ranging from 0 where no symptoms appeared to 100 where all plants were severely affected. Indices for the varieties tested are in Table 2, where it is shown that Badger Market is equal to the parents in resistance to mosaic and is distinctly more resistant than Resistant Detroit and Wisconsin Golden Acre.

[5] Boswell, V. R., W. C. Edmundson, O. H. Pearson, J. E. Knott, C. E. Myers, R. A. McGinty, W. H. Friend, H. H. Zimmerley, and J. C. Walker. 1934. Descriptions of types of principal American varieties of cabbage. U. S. Dept. Agr. Misc. Publ. 169.

TABLE 2.—*Mosaic indices of Badger Market, in comparison with certain other standard varieties, Madison, Wis., 1952*

Variety	Mosaic index
Badger Market	48
Racine Market	46
Globe	48
Medium Copenhagen Market Resistant	50
Marion Market	52
Resistant Detroit	60
Wisconsin Golden Acre	65

SUMMARY

A new variety of cabbage, Badger Market, is described. It is a round-head, second-early type bred to produce small compact heads to meet current fresh market needs. It is highly resistant to yellows and moderately resistant to mosaic.

DIVISION OF VEGETABLE CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION
U. S. DEPARTMENT OF AGRICULTURE
AND
DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

MEASUREMENT OF RESISTANCE IN ALFALFA TO COMMON LEAF SPOT [1]

Fred Reuel Jones

Resistance in alfalfa to common leaf spot caused by *Pseudopeziza medicaginis* (Lib.) Sacc. has been under study since resistant plants were reported in 1941.[2] Plants selected that year, and later, have been bred to combine this resistant character with other desirable characters which some of the original selections lacked. At first, resistance was recorded from behavior in the field. When years with little leaf spot halted progress by this method, inoculations of seedlings in the greenhouse with pure cultures became standard procedure. Finally it was found that from an examination of the early development of the mycelium of the fungus in the leaf tissue it was possible to classify seedlings much earlier and to identify the resistant character in older plants more economically than by previous methods. This classification of seedlings can be used as a technical measure of resistance in a variety. A description of this method and some of the first results obtained with it follow.

MAINTENANCE OF PURE CULTURES.—Since the maintenance of pure cultures of the pathogen with abundant fruiting has often given trouble, improvements in the methods reported in a previous paper[3] have been sought. Although the fungus will fruit occasionally on a variety of vegetable media, oat agar has been the most dependable. Cooking the agar with the oatmeal for at least an hour before autoclaving appears to improve it, and the use of 2 per cent agar is preferred to the 1 and one-half per cent usually recommended. The oat agar has been supplemented to advantage by the addition of about 20 per cent of a malt agar prepared with 2 per cent of malt sprouts. Spores are discharged upon this agar combination, but after 10–15 days, or when the fungus crust can be cut into strips with little agar adhering, this crust is transferred to agar slopes without the added malt. The spore discharge upon the agar slope should be adequate in 4–5 days. The best temperature for the maintenance of cultures is 65–75°F., and temperature above 75° for an extended time appears distinctly injurious. Fruiting is abundant in about a month and is often maintained at a high level for 2 weeks.

Cultures isolated thus far have deteriorated in spore producing ability after 2 years. Deterioration is first indicated by failure of the ascospores to discharge and germination in the ascus. Thus it has been found expedient to make 1 or 2 new isolations each year. No alteration of pathogenicity with age in cultures has been detected. Since it might be assumed that cultures from resistant plants might have higher pathogenicity than those from susceptible plants, 1 such isolation has been compared and found no more pathogenic than 2 others from susceptible plants.

PROCEDURE IN INOCULATION.—Inoculation for test of resistance is made either upon single leaves on cuttings of older plants, or upon the first unifoliate leaf of seedlings. In any case, inoculation is made in petri dishes poured with a thin layer of very clear agar. A culture actively discharging spores is transferred to the cover of the dish. Beneath it are placed the leaves on cuttings or the first leaf of the seedling as soon as it is fully expanded. The seedlings are grown in sand culture from which they are removed, and the leaf is pressed lightly on the agar to which it will then adhere during spore fall. The amount of spore fall can be judged from time to time by inverting the petri dish under the low power of the microscope for observation of spores on the agar in spaces left between leaves. When sufficient spores have fallen—4–12 hours may be required—the cuttings or seedlings are removed to a moist chamber for at least 12 hours before setting in the greenhouse, and the culture can be placed over more leaves. Cultures can often be used for over a week in this manner. Inoculation and moist chamber incubation have all been maintained at 65–70°F.

In the greenhouse precautions must be taken to prevent wilting of inoculated leaves, and temperature should not rise higher than 75°F. for any considerable period. In 7–8 days the fungus indicates by its development the degree of resistance that exists in the inoculated plants.

PREPARATION OF LEAVES FOR MICROSCOPIC OBSERVATION.—In seedlings and in most plants grown in the greenhouse, the early development of the parasite in leaves can be observed directly after the leaf has been boiled in equal parts of glacial acetic acid and absolute alcohol. When large numbers must be compared, this procedure is necessarily employed. Leaves from plants in the field are often too thick to permit this method, and in such cases and for many details of host-parasite relations the material has been imbedded and stained.

GENERAL FEATURES OF INFECTION.—In the observation of infection, 3 features are common in all plants observed thus far. First, the penetration of epidermal cells has been observed in all plants. In some highly resistant plants it appears that under some circumstances fewer spores penetrate than in susceptible plants, but when spores are abundant some always enter. Thus failure to penetrate the host in some degree has not been found a useful index of resistance. Second, the penetrated cell is seen as more or less yel-

[1] Accepted for publication August 3, 1953.
Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] Jones, F. R., Allison, J. L., and Smith, W. K. 1941. Evidence of resistance in alfalfa, red clover and sweetclover to certain fungus parasites. Phytopathology 31: 765-766.

[3] Jones, F. R. 1919. The leaf-spot diseases of alfalfa and red clover caused by the fungi Pseudopeziza medicaginis and Pseudopeziza trifolii, respectively. U. S. Dept. Agr. Bul. 759. Professional Paper.

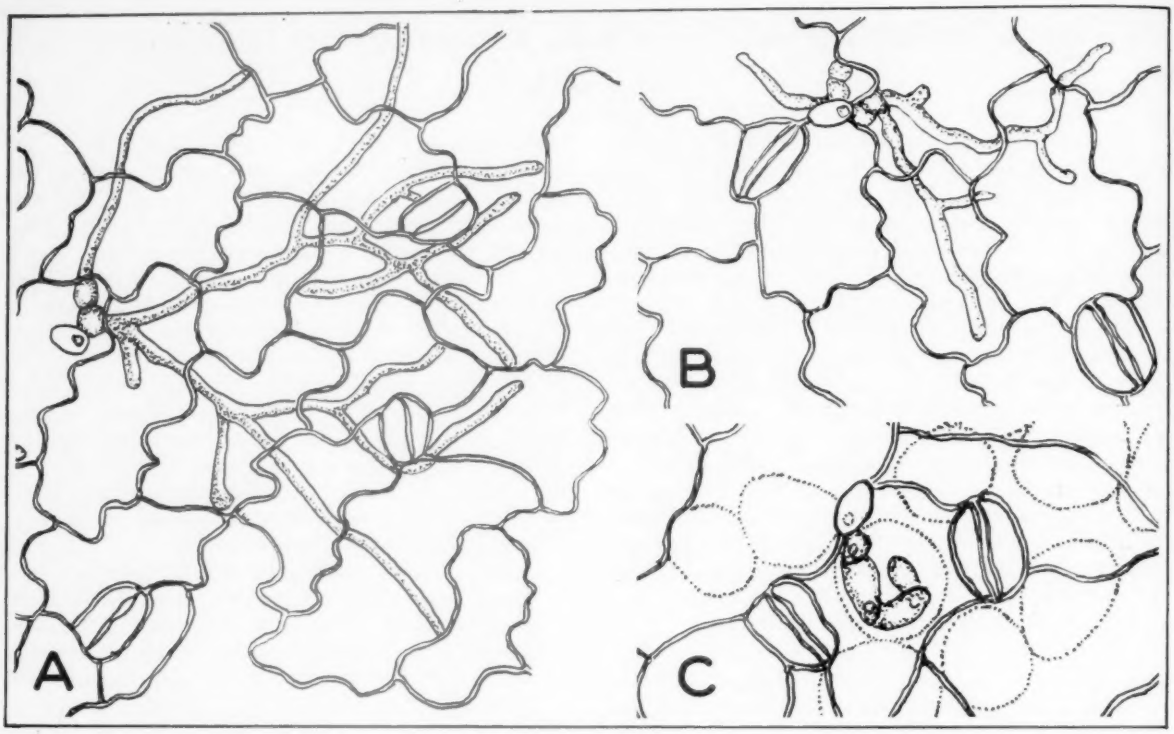

FIG. 1. Typical development of the mycelium from a germinating spore of *Pseudopeziza medicaginis* after 7 days in leaf tissue of plants classed as A) susceptible, B) moderately resistant and C) highly resistant. For further explanation see text. ×700.

low in color in cleared leaves. This circumstance makes penetrated cells easy to locate. The amount of yellowing differs greatly in plants, from very faint to so deep that the growth of the fungus in the cell is not easily observed. Usually surrounding cells entered by the fungus are not immediately yellowed, but there is great variation in this behavior. The extent and degree of early yellowing after penetration does not appear to be correlated with resistance. Third, the early growth of the fungus in the host is strictly intracellular. In fact, intercellular spaces appear to impede the growth of the fungus. Later when the fruiting stroma begins to form in susceptible plants the fungus passes readily through intercellular spaces.

MICROSCOPIC EVIDENCES OF RESISTANCE.—In completely susceptible leaves the pattern of growth of the fungus is simple and uniform. The mycelium branches in the first epidermal cell entered and radiates usually in all directions through surrounding cells. Cell walls appear to offer no obstruction to growth. The extent and character of this growth is shown in Fig. 1, A. Usually few branches have penetrated downward into the palisade layer at this time, though shortly afterward the entire mesophyll becomes filled with the fungus which spreads again in the lower epidermis, though not so far as in the epidermis first entered. In 2 weeks the stroma in which the apothecium will develop is formed at or near the point of infection.

In leaves which are classed as the most resistant found thus far, the behavior of the fungus is equally uniform and strikingly different. The penetrating germ tube does not branch and spread in epidermal cells, but if it survives after penetrating, it passes directly into a palisade cell. Here growth is slow, and at the end of a week it may not have passed beyond the first palisade cell entered (Fig. 1, C). In such plants the palisade cells appear to be a little less resistant than the epidermal cells, but in no case do they completely inhibit growth of all the penetrating germ tubes. Plants do not inhibit the penetrating fungus equally, and thus a subdivision of this class of plants seems possible, though probably not useful at present.

Between the extremes of resistance and susceptibility are plants distinguished by a slower growth of the entering fungus than in completely susceptible plants and usually by lack of the uniformity of the direction of growth taken in highly resistant plants. Sometimes the only distinguishing character is less growth of the same character as in susceptible plants (Fig. 1, B). But more often the character of growth differs. Fungal cells may be short and swollen, or mycelial strands may lie close together, or many short cells like conidia may be produced. When growth is highly restricted many of the germ tubes may pass directly to the mesophyll while a few grow a short distance through the epidermis. Obviously this class contains plants which differ all the way from very low to

very high resistance, but no precise method of subdividing it has become evident. In making selections it is often important to note whether a plant reaction lies near the upper or the lower margin of the class.

CLASSIFICATION OF PLANTS.—From the observation of the early development of the fungus in the host 3 classes of plants have been distinguished. First are those completely susceptible, in which the mycelium first spreads through epidermal cells at approximately the same rate and the same manner from all spores that send a germ tube through the epidermis. Second is a resistant class characterized by a slower rate of growth of the fungus in the host with diverse characteristics of growth. Third is a highly resistant class in which there is no spread of the fungus in the epidermis, but a direct entry through the epidermis to the mesophyll in which the fungus may grow slowly, or scarcely any at all, without fruiting.

The time consuming labor in classifying plants consists in the microscopic examination of the decolorized leaves, which appears essential with more resistant plants. If non-resistant plants are numerous in a population they can usually be recognized by macroscopic appearance after experience.

This classification does not appear at present to be a suitable basis for a genetic study of resistance, partly because it attempts to deal with 2 sets of variations. In the host, resistance in the mesophyll always lags behind that in the epidermis, but not equally in all plant progenies. Thus when early growth is only in the mesophyll it may in time give rise to conspicuous black spots in some plants of this class but not in others. However, judging from field experience thus far it appears that such spots develop so slowly and are so few in number that they are not likely to be of economic consequence. The other set of variations appears to be in the fungus as judged by its various development in plants of low resistance. Since fruiting cultures of the fungus have not yet been developed from single spores, a study of this source of variation has not been possible.

The advantage of this method of classification over that devised by Davis [4] lies in the possibility of determining the resistance of any plant in field or greenhouse in about 10 days by the use of cuttings, and in the early elimination of the susceptible fraction in a population of seedlings. The class of highest resistance by this method apparently includes class 1 and a part of class 2 of Davis, intermediate resistance includes the rest of class 2 and class 3, and susceptible includes classes 3 and 4.

EFFECT OF ENVIRONMENT UPON RESISTANCE.—The method of inoculation described offers opportunity for the study of the effect of environment upon infection, but thus far no important modifying factor has been found. The age of the leaf and culture solutions in which the cutting is placed have not been found to modify infection appreciably thus far. Infection proceeds in the dark as well as in light so long as the leaf remains alive. From field observations it appears that foliage produced in long continued sunny weather in spring is more resistant than that produced in the greenhouse, but this is not yet confirmed experimentally.

HOW RESISTANT CAN A VARIETY OF ALFALFA BE MADE?—The only leaf spot resistant variety known to the writer at present is Caliverde. Three hundred fifty seedlings of this variety have been inoculated in the unifoliate leaf and classified in the manner here described. Thirty seven per cent were placed in the most resistant class, 18 per cent did not appear to have resistance, and the remaining 45 per cent were in the intermediate class with most of the plants near the upper margin of the class. A part of these plants were inoculated later as cuttings with substantially the same result. From experience with experimental stocks now under study it appears that a higher standard than this may be attained if it is desirable. Selfed progenies have been obtained containing 85 per cent of plants in the most resistant class, and none completely susceptible.

SOURCES OF RESISTANT PLANTS.—The leaf spot resistant plants that have been used thus far in alfalfa improvement and study have been chance finds in fields or have been noted in nurseries of clones where the character is more easily observed. In common varieties such plants appear to be rare. In a search for resistant plants in the variety Narragansett nearly 600 seedlings were inoculated and about 10 per cent were judged to have some degree of resistance, though only about 1 per cent were in the most resistant class. On the other hand, a selection of alfalfa made some years ago by A. R. Albert at Hancock, Wis. from a field of presumable Grimm, and which has been grown on a small scale locally, was found to have 75 per cent of plants with some resistance, and 25 per cent in the highest class. Resistant plants have been found in Ranger and in Buffalo. Thus it appears that resistant plants are accessible in the common varieties, and the increase of that character in a variety offers no more difficulty than increase in resistance to bacterial wilt.

SUMMARY

Alfalfa plants resistant to common leaf spot have been found in several varieties, and this resistance has been studied in populations obtained by selfing and crossing such plants. A rapid method of estimating resistance has been devised by the observation of the growth of the fungus in the leaf tissue in decolorized leaves 7–8 days after inoculation. The method is equally applicable to the first leaf of seedlings or to leaves on cuttings of older plants grown in the greenhouse. Three classes of plants with reference to resistance are recognized. In completely susceptible plants the fungus spreads first through epidermal cells. In the most resistant class there is no spread of the fungus in epidermal cells, but the penetrating germ

[4] Davis, R. L. 1951. A study of the inheritance of resistance in alfalfa to common leaf spot. Agron. Jour. 43: 331-337.

tube, if it survives, passes directly into the mesophyll where it may develop closely packed mycelium very slowly without eventual fruiting. In the intermediate class, the fungus may spread very slowly in the epidermis, often showing striking malformation.

The size of these 3 classes of plants in a variety is suggested as a measure of resistance.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

FACTORS AFFECTING THE PARTIAL VACUUM INOCULATION OF SEEDLING CORN WITH USTILAGO ZEAE[1]

J. B. Rowell and J. E. DeVay

A quick and reliable method is needed for determining pathogenicity and sexual compatibility among biotypes of *Ustilago zeae*. It is necessary to inoculate active corn meristems with suitably paired haploid lines or with diploid lines to obtain galls and chlamydospores. Stakman and coworkers (4) inoculated 2–4-week-old plants by hypodermic injection of inoculum into the growing point area of the stem. The number of compatibility tests made by this method is limited by the available greenhouse space for growing plants, a disadvantage eliminated by Stevens et al (5) who injected the inoculum directly into the plumule of germinating corn seedlings. However, hypodermic methods require repeated sterilization of equipment when many different smut lines are tested. A partial vacuum was used by Wilkinson and Kent (7) to force smut inoculum into the leaf whorl of corn seedlings in the fourth-leaf stage, and by Wallin (6) to inoculate wounded seed covered by a suspension of inoculum. Wallin's technique is simple, but a low percentage of infection usually results. A modification of these methods has been developed (3) and used during the past 2 years in 14,967 tests of pathogenicity and sexual compatibility of 2,482 isolates and mutants of *Ustilago zeae*.

The present paper describes the role of critical factors affecting the partial vacuum method of inoculating corn seedlings. The application of the method to determine quantitative and qualitative differences in the pathogenicity of the cross between haploid lines 10A4 and 17D4 and the diploid progeny from this cross, 410qq and 410n, is also described.

METHOD.—Seedlings are obtained from surface disinfected corn seeds which are germinated aseptically until the coleoptile is approximately 1 cm. long. Two mm. of the coleoptile tip are cut off to open the cylinder of tissues that surrounds the plumule and growing point. Twenty to 30 seedlings are placed in a 6 × 1 in. test tube and covered with a cell suspension of the lines to be tested. The tubes are placed in a chamber and exposed to the maximum partial vacuum obtained with a water aspirator for 5 minutes. The inoculum is drained from the tubes after this treatment, and 10 seedlings per replicated pot of sterile soil or vermiculite are planted. Appropriate precautions are employed throughout the procedure to prevent cross contamination of the inoculum. Two workers with this method can inoculate seedlings with 300 different treatments or crosses in 6–8 hours.

FACTORS INFLUENCING DISEASE DEVELOPMENT.—Certain factors are important for obtaining uniform results with this method. Inoculated seedlings must be free from seed-borne organisms to prevent preemergence killing after planting. The tip of the coleoptile must be cut off for effective inoculation. Galls developed in no more than 10 per cent of uncut seedlings and in 100 per cent of cut seedlings after inoculation with compatible, virulent lines. The protection of the meristems by the coleoptile cylinder was demonstrated by exposing wounded and nonwounded seedlings, in a suspension of India ink, to a partial vacuum. The ink particles penetrated and blackened the coleoptile only in the decapitated seedlings (Fig. 1A). The tissues between the coleoptile and scutellar nodes were heavily permeated by the ink particles in seedlings of both treatments, but no symptoms of infection have ever been observed in these tissues of inoculated seedlings.

Best results were obtained with seedlings having a coleoptile 0.5–1 cm. long. The inbred dent corn line, B 164, used in these tests required 48–72 hours at 27° C. to grow to this size; 100 per cent infection was obtained with such seedlings. Only 45 per cent infection was obtained with seedlings germinated for 24 hours prior to inoculation. A high percentage of 4-day-old seedlings became infected but the long radicles and coleoptiles made such seedlings difficult to handle without breakage. Furthermore, the type of gall development obtained with these large seedlings was less uniform than with 42–78 hour-old seedlings.

Rate of development of galls in the inoculated corn depends on the temperature at which the seedlings are grown after inoculation. Table 1 compares the percentage of infected plants with galls at various times during incubation at 3 temperatures of the cross between the haploid lines, 10A4 × 17D4, and the 2 solopathogens, 410qq and 410n. Well-developed galls were formed in 100 per cent of the plants within 10–11 days at an approximate temperature of 85° F., following inoculation with the compatible haploid lines. At 70° F., 16 days were required and at 60° F., 24 days. The 2 solopathogens produced galls more slow-

[1] Accepted for publication August 10, 1953.
Research conducted under contract number AT (11-1)-42 between the U. S. Atomic Energy Commission and the University of Minnesota.
Paper No. 3002, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.

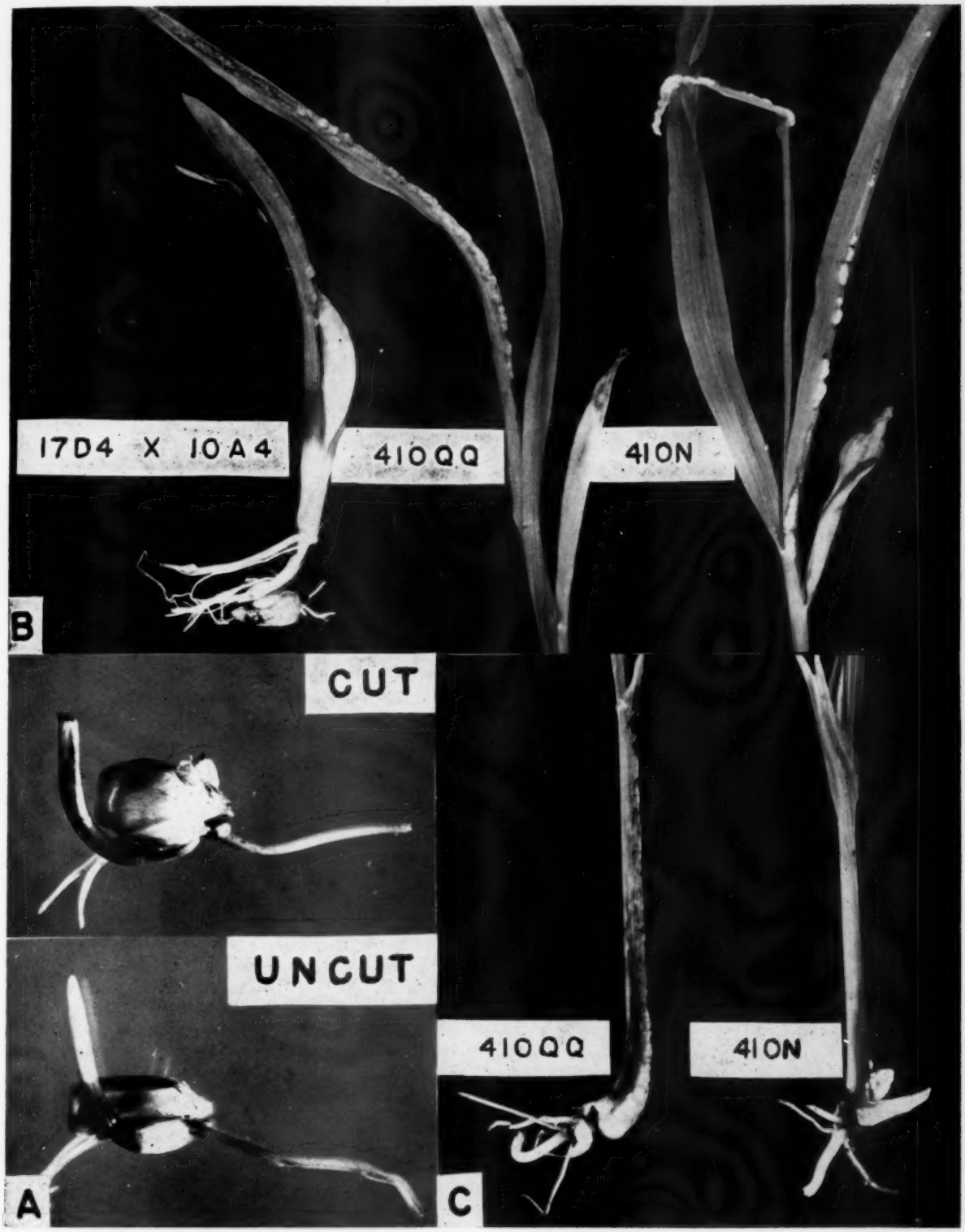

FIG. 1A) Penetration of India ink particles in the coleoptile of uncut and cut corn seedlings (2× normal size); B) Characteristics of infection in 4 lines differing in pathogenicity. Lines 10A4 × 17D4, 10 days after inoculation, 410qq and 410n, 18 days after inoculation. Note presence of anthocyanin pigments around 410qq galls and absence in 410n infections. C) Characteristics of stem infections in lines 410qq and 410n 18 days after inoculation.

TABLE 1.—*Effect of temperature on the rate of development of galls in corn seedlings inoculated by the partial vacuum method*

Temperature	Inoculum	Percentage of Infected Plants With Galls: Days after inoculation 11	16	20	24
60° F.	Control	0	0	0	0
	17D4 × 10A4	27	76	94	100
	410qq	0	0	38	54
	410n	0	0	3	12
70°	Control	0	0	0	0
	17D4 × 10A4	92	100	100	100
	410qq	0	3	47	89
	410n	0	8	8	48
85°	Control	0	0	0	0
	17D4 × 10A4	100	100	100	100
	410qq	25	83	100	100
	410n	0	18	73	73

ly, requiring 7–9 additional days. Some plants inoculated with these lines failed to produce galls although anthocyanin spotting in the leaves and stem indicated that infection had occurred. Apparently the effect of temperature on the rate of gall development under these conditions was directly correlated with the rate of growth of the corn plant, i.e., the more rapid the rate of plant growth, the more rapid the development of the galls.

The growth type and number of cells used for inoculation affect the incidence of disease in seedlings. Poor infection was obtained with lines which grow as mycelial clumps in broth unless the inoculum was macerated with a Potter tissue crushing homogenizer or a semi-micro Waring Blendor. Inoculum prepared from cells of a sporidial growth type produced the most uniform infections and for this reason a sporidial mutant (17D4-C1) was used in most of these tests. Experiments to determine the role of the number of cells per ml. of the inoculum (inoculum density) in disease development were made with lines that grow as individual cells in broth shake-cultures. Table 2 lists the percentage of infected plants at different inoculum densities of 10A4 × 17D4-Cl, 410qq, and 410n. Only an occasional seedling escaped infection above 5 million cells per ml.; with fewer cells, the proportion of escapes increased rapidly. The number of cells that reached the susceptible tissues was limited by the small liquid volume that penetrated to the infection court within the coleoptile cylinder. The penetrating volume apparently lacked an effective number of cells for initiating infection when the concentration of cells in the inoculum became too low.

TABLE 2.—*Effect of inoculum density on percentage of infection of corn seedlings inoculated by the partial vacuum method*

Cells in Inoculum (millions/ml.)[a]	Percentage Infection: 10A4×17D4-Cl	410qq	410n
50	98	100	98
10	98	99	98
5	90	97	100
1	82	89	88
0.5	72	77	74
0.1	34	61	48

[a] The 10A4 × 17D4-Cl cross had this number of cells for each line.

FIG. 2. Effect of inoculum density on severity of disease in seedling corn inoculated by the partial vacuum technique. Left to right 0.1, 1, 10, and 100 million cells per ml. in inoculum.

The severity of disease was also directly dependent on the number of cells per ml. in the inoculum. Fig. 2 shows the progressive increase in disease effects from 0.1 to 100 million cells per ml. for 3 different pathogenic entities (10A4 × 17D4-Cl, 410qq, and 410n). Although there was a marked difference in plant height between these 2 extremes, this difference was not readily apparent in the fresh weight of the plants. Dwarfing of the more severely diseased plants was offset by the increase in stem volume from the galls. The percentage of plant tissue involved in galls was calculated from the total fresh weight of the stem and leaves and the weight of the gall tissue after excision of unaffected tissues. The effect of the number of cells in the inoculum on the percentage of gall tissue is shown by a semilogarithmic plot in Fig. 3. There was no change in the gall type under these conditions although there was a consistent increase in the amount

of gall tissue with increasing inoculum concentration.

CHARACTERISTICS OF CORN SMUT IN SEEDLINGS.—The foregoing data have demonstrated measurable differences in the disease resulting from inoculation with the cross between lines 10A4 × 17D4 and the 2 diploid lines 410qq and 410n. Larger galls were consistently formed in plants inoculated with the cross between the haploid lines than in the diploid lines (Fig. 2 and 3). There was also a slight but constant difference between the 2 solopathogens in the amount of gall tissue formed, 410qq developing the larger galls. Data in Table 1 illustrate the different rates of gall development for the 3 pathogenic inocula.

Symptoms of infection were first apparent in the cross between the haploid lines 10A4 × 17D4 (or the sporidial mutant 17D4-Cl) 5 days after inoculation at an incubation temperature of approximately 85° F. The first symptoms were chlorotic flecks on stems and leaves, and galls appeared 2 days later. Maximum development of the galls occurred within 10 days after inoculation and generally the entire stem area was involved (Fig. 1B). The galls in most crosses that had been tested by this method became filled with chlamydospores in the next 4 days. However, chlomydospore formation was suppressed in all crosses involving the line 10A4. The galls generally were invaded and rotted by secondary organisms and the seedlings killed during the third week after inoculation.

The solopathogenic lines, 410qq and 410n, originally were isolated from chlamydospores of the cross 10A4 × 17D4 but differed markedly from the parent cross in rate of development of disease symptoms. The chlorotic flecks were first apparent after 8 days' incubation at 85° F.; galls appeared in 10 days and reached maximum development 18 days after inoculation. Most galls were formed on the leaves and infrequently occurred on the stem. These diseased plants were distinctly different in appearance from the plants inoculated with 10A4 × 17D4 (Fig. 1B and 1C). Furthermore, disease development was more rapid and vigorous for the line 410qq than 410n. Anthocyanin pigments were present around the galls produced by 410qq but were absent in 410n infections. The 2 lines also differed in the type of stem gall that was formed when such development occurred (Fig. 1C). The galls generally were formed along 1 side of the stem in 410qq infections and extended from the coleoptile node up into the leaf sheath. However, in 410n the galls were restricted to the area of the coleoptile node and appeared to develop from an adventitious root initial. Few chlamydospores were obtained from these galls; presumably because of the presence of the dominant factor from the 10A4 parent which suppressed their production. The differences in disease development outlined were consistent in tests with 20 corn lines, crosses, and varieties representing extremes of field resistance and susceptibility.

Cross inoculations of the 4 lines were made repeatedly in attempts to alter the characteristic differences in disease reaction. In such tests, uniform seed-

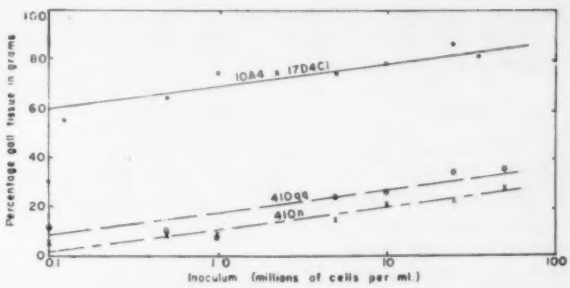

FIG. 3. The percentage of gall tissue produced in corn seedlings inoculated by the partial vacuum technique with *Ustilago zeae* inoculum of varying cell concentration.

lings of the inbred dent corn line, B 164, were inoculated by the partial vacuum technique with all possible combinations of the lines 10A4, 17D4, 410qq, and 410n in distilled water. The inoculum density was 10 million cells per ml. for each line in a treatment. Eight randomized, replicated pots of 10 seedlings each were planted per treatment and were maintained at an approximate temperature of 85° F. Four pots per treatment were harvested by cutting the plants at the coleoptile node for the data after the 2 growth periods. All plants were measured as individuals, but all plants and galls from a single replicate were weighed simultaneously. The plants inoculated with the cross of lines 10A4 ×17D4 alone and in combination with other lines were not measured because of the distortion and rigidity which results from the more severe gall development. Table 3 records the differences in plant length, fresh weight, and percentage of gall tissue for these combinations.

Combinations of the 3 pathogenic entities with additional lines generally resulted in a less virulent reaction. Plants inoculated with either line 17D4 or 10A4 were stunted. This reaction was most pronounced for the former line. The curl reaction reported by Munnecke (2) in larger plants inoculated by the hypodermic technique with certain lines including 17D4 has not been observed in the present tests. However, small necrotic flecks were present on the tips of the leaflets of the stunted plants inoculated with 17D4, and this line was reisolated from these areas when surface sterilized leaf tips were plated on water agar. Neither of the haploid lines when used in a combined inoculum with the 2 diploid lines materially increased the disease reaction to a level comparable with the cross 10A4 × 17D4. Marked stunting and a reduction in galls was noted in the combination 410qq and 17D4. Both the diploid lines retarded the degree of gall development in the cross of lines 10A4 × 17D4. The combination of the 2 diploid lines also produced a less severe disease reaction than that of 410qq alone, the more virulent of these 2 lines. Such reduction in the severity of disease with composite inocula of haploid lines of different virulence has previously been reported by Kernkamp and Martin (1).

The differences in pathogenicity between the cross of lines 10A4 × 17D4 and the solopathogenic diploid

TABLE 3.—*Differences in plant and gall size in seedlings inoculated with various combinations of the 2 haploid smut lines, 10A4 and 17D4, and their diploid progeny, 410qq and 410n*

Inoculum	Mean Plant Length (cm.)		Mean Fresh Weight (g.) for 10 plants		Percentage of Plant Weight Galled	
	10 Days	17 Days	10 Days	17 Days	10 Days	17 Days
Uninoculated	21.6	34.9	7.1	13.2	0	0
17D4	16.5	25.1	5.2	10.1	0	0
10A4	18.3	30.1	5.9	11.9	0	0
10A4 × 17D4	----	----	4.1	3.3[a]	70.8	74.3[a]
10A4 × 17D4 × 410qq	----	----	4.0	4.4[a]	65.0	58.6[a]
10A4 × 17D4 × 410n	----	----	4.3	4.1[a]	48.9	55.8[a]
410qq	15.3	18.2	5.8	5.6	8.5	33.9
410qq × 17D4	13.0	13.6	4.9	5.2	4.1	27.4
410qq × 10A4	14.9	16.5	5.7	5.8	5.3	37.3
410n	15.9	22.8	5.5	10.0	Tr	17.2
410n × 17D4	14.5	22.6	6.0	9.5	Tr	22.5
410n × 10A4	16.9	22.6	6.5	10.3	Tr	25.6
410n × 410qq	15.4	21.5	5.8	9.1	Tr	25.8

[a] Data taken after 12 days' incubation as plants were dying from secondary infections.

lines obtained from this cross are difficult to explain. Genetically, the diploid lines should be identical to the parental cross. The pathogenic differences might be due to chromosomal aberrations or mutations. However, a disease reaction similar to that produced by 10A4 × 17D4 was obtained in all compatible matings of a limited number of segregants obtained from chlamydospores of line 410qq. Furthermore, some solopathogens recently isolated from 10A4 × 17D4 chlamydospores also produced a less virulent disease reaction in infected seedlings. Apparently such a reaction is an expression of the solopathogenic condition obtained from the 10A4 × 17D4 genetic entity.

SUMMARY

A rapid and efficient method for determining the characteristics of pathogenic and sexual compatibility in mutants and isolates of *Ustilago zeae* consists of cutting the coleoptile tip from germinating seedlings, immersing the seedlings in the inoculum, and subjecting the material to a partial vacuum. The seedlings are planted after this treatment and galls and chlamydospores normally develop within 2 weeks. The essential factors for high percentages of uniform infection are: 1) uniform aseptic seedlings with a coleoptile 0.5-1 cm. long; 2) cut tips of the coleoptiles to allow even penetration of the inoculum to the growing point and plumule; 3) an inoculum in the form of a suspension with at least 1 million cells of each line per ml. Galls and chlamydospores form most rapidly at an incubation temperature of approximately 85° F., and form progressively slower at 70° and 60° F.

Differences in the amount of gall formation were measured quantitatively by calculating the percentage of gall tissue from the fresh weight of plants above the coleoptile node and of the excised galls. The cross of lines 10A4 × 17D4 consistently resulted in a larger percentage of gall tissue in infected plants at all inoculum levels than the 2 solopathogens, 410qq and 410n. Furthermore, 410qq produced slightly larger galls than 410n. The infections produced by 10A4 × 17D4, 410qq and 410n differ qualitatively and quantitatively in the rate of development of infections, the appearance of stem galls, and the production of anthocyanin. The differences in these characteristic infections could not be altered by the number of cells in the inoculum or by different corn hosts. Furthermore, mixtures of the 4 lines in all possible combinations failed to produce a major change in the infection type although generally the severity of infection was depressed by such mixtures.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
UNIVERSITY OF MINNESOTA
UNIVERSITY FARM, ST. PAUL 1, MINNESOTA

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SOME CHEMOTHERAPEUTIC AGENTS FOR WHEAT STEM RUST [1]

Hugh Howison Hotson

The degree of susceptibility or resistance of wheat varieties to a particular group of biotypes of stem rust has been shown to be relatively constant under controlled environmental conditions. Stakman and Piemeisel (9) have shown conclusively not only that the concept of physiologic race based on the interaction of host and rust is a valid criterion of clonal pathogenicity, but that within certain environmental and nutritional limits this interaction is concise and definite. Thus, if this interaction of host and rust can be changed by applying a chemical after infection has taken place, the resulting change in the type of rust-host reaction would be due to the chemical applied.

Gassner and Hassebrauk (1) tested about 300 chemicals for the therapeutic effects on cereal rusts. They applied these chemicals to the soil at the time that the plants were infected and noted the change in rust reaction. The most promising chemicals were acridin, 1-bromo-2-napthol, alpha-napthol, nitrodibrombenzol, picric acid, para-toluol-sulfochloramide-sodium salt, and 3, 4, 6 tribrom-1, 2-napthoquinone. Continuing this research, Hassebrauk (3) found that the most effective chemicals used by Gassner and Hassebrauk were picric acid, trinitrotoluene, p-toluolsulfochloramide-sodium salt and in addition p-tosuolsulfonamide. All were effective when applied to the soil in large quantities. Hart and Allison (2) controlled stem rust by soil application of sodium borate (Borax—sodium tetra borate—$Na_2B_4O_7$), and picric acid, para and ortho toluolsulfonamides. Their results, though good, required about 80 lbs. of chemicals per acre.

Hassebrauk (4) next directed his attention to chemicals against 4 rusts—*Puccinia triticina*, *P. coronata*, *P. simplex*, and *P. glumarum*. The following chemicals gave good control when applied to the soil at the time of inoculation: para-amino benzol sulfonamide, acetyl sulfanilamide, 2-para-amino benzol sulfonamido-pyridine, 6-sulfanilamido-2, 4-dimethyl pyridine, and saccharin.

T. Johnson (7) obtained an increased susceptibility by spraying Khapli emmer with DDT. Khapli is very resistant to all known races of stem rust, except 42, 72, 99 and 189. When Khapli wheat was sprayed with DDT after inoculation with race 17 of wheat stem rust, susceptible (3-4) type pustules developed.

Chemicals used to control wheat stem rust must share certain characteristics with those chemicals used to control human and animal diseases. Such chemicals to be effective chemotherapeutic agents, must be able to enter the host and reach the site of infection at a concentration sufficiently high to prevent the pathogen from developing further. If these chemicals are toxic to the host, their concentration must allow a margin of safety between the therapeutic dose and the phytotoxic dose. This margin of safety is also involved when one is seeking metabolic intermediates by reversible inhibition techniques.

MATERIALS AND METHODS.—This report deals entirely with data obtained in greenhouse experiments.

The wheat (Little Club, *Triticum compactum* Host.; Spelmar, *T. durum* Desf.; Vernal and Khapli emmers, *T. dicoccum* Schr.) seedlings were thinned to 10 plants per 4 in. pot and 1 week after planting were inoculated with 1 of 4 races (15B, 17, 38 or 56) of wheat stem rust (*Puccinia graminis tritici* Erikss and Henn).

Inoculation procedures.—The uredospore inoculum was mixed with talc USP, 1 part of uredospores to approximately 100 parts of talc. This mixture provided a medium-to-heavy dose of inoculum and a relatively uniform spore distribution. Each primary leaf developed at least 1 uredium; the average number per primary leaf was 6–12. Only occasionally was the inoculum so dense and the leaves so heavily infected that the mycelium joined in adjacent pustules. The use of approximately .01 gm. of spores for 100 pots of wheat seedlings resulted in adequate coverage. Standard cylindrical inoculators were used in a room in which the temperature was controlled at 17°C. Warm water was added to the base of the incubator to help form dew on the leaves. The potted plants, thinned to 10 plants per pot, were placed in an incubator. The uredospore-talc mixture was then dusted evenly over the leaves with a hand duster and the cover placed on the incubator. Plants were inoculated in the afternoon and were removed to the greenhouse the next morning, after aproximately 18–20 hours of incubation.

Selection of chemotherapeutic agents.—Soil application of the chemicals allowed a very narrow margin of safety between a concentration suppressing rust development and a concentration toxic to the host plant. In addition, the rates of application were high (about 80 lbs. per acre). A more economical test method was needed and a chemical which could be applied after infection had taken place.

The soil application method was used first to obtain a chemical which would suppress rust development. The chemical to be tested was dissolved in water, or in Tween-20 if it had a low solubility in water, in sufficient quantity to allow 50 ml. of chemical per 4 in. pot in the experiment. The test concentration was 0.5 ml. of chemical per 4 in. pot in 50 ml. of solution (rate about 70 lbs. per acre). Of the newer sulfonamides tested (i.e., sulfanilamide, sulfathiazole, sulfamerizine), sulfamerizine gave excellent control of stem rust development at the rate of 4 mg. per pot (about 55 lbs. per

[1] Accepted for publication Aug. 10, 1953.
Paper No. 2960 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.
A portion of a thesis submitted to the Graduate School, University of Minnesota, in partial fulfillment of the requirements for the Doctor of Philosophy degree (1951).

acre) when applied in aqueous solution to the soil.

Application of chemical to test plants.—Using sulfamerizine as a test chemical for the effectiveness of treatment, experiments on the method of applying the chemical were made. After several experiments with sticker spreaders and immersion treatments, a spray treatment was found to give most uniform results when a wetting agent, Tween-20, was added.

Tween-20 (polyoxyethylene sorbitan mono-laurate), manufactured by the Atlas Powder Company, was found to be neither toxic to the wheat nor toxic to the uredospores of the rust at a concentration of 3 per cent by volume. A 10 per cent solution in distilled water has a pH of 7. Tween-20 aids in the penetration of chemicals into leaves at 1 per cent and 0.5 per cent of Tween-20 by volume. Slightly better results were obtained with a 1 per cent solution of Tween-20 and this concentration was adopted as a standard ingredient for spray mixture.

The rate of application of chemicals required for the successful control of stem rust was reduced from about 80 lbs. per acre to about 14 lbs. pre acre by the use of sulfamerizine spray with 1 per cent Tween-20 added.

The experimental schedule consisted of this pattern: seeds planted—0 days; seedlings thinned to 10 per pot and inoculated with stem rust—7 days after planting; removed from incubator to greenhouse bench—8 days after planting; 20 ml. chemical (300 p.p.m.) sprayed onto 60 plants in 6 pots—10 days after planting; reaction noted—21 days after planting; reaction noted again—25 days after planting; pots discarded—30 days after planting.

The spraying mechanism consisted of a compressor and a glass atomizer or de Vilbis atomizer. The compressor used was a combination compressor—vacuum pump with a capacity of 27 in. of mercury driven by a ¼ hp. A.C., 110 V., 60 cycle Westinghouse motor. This is a compact unit manufactured by the Gast Manufacturing Corp., Benton Harbor, Michigan, weighs 40 lbs., and is portable.

The 20 ml. sprayed over 6 pots of wheat gave a coverage which just reached the run-off stage and was used as a standard volume dosage.

After standardization of this technique, additional races of stem rust were included as experiments were repeated. Each experiment was repeated 3 times and was replicated 3 times with 10 plants per pot.

Results.—The sulfa-drugs as a group were tested by the standardization method. The sodium salt of sulfadiazine was most effective in suppressing rust development; however, sulfapyrazine, sulfapyridine, and gantrisin (3, 4, dimethyl-5-sulfanilamide-isoazole) were almost as effective as sulfadiazine. A comparison of the effectiveness of these compounds is in Table 1. No difference could be noted in effectiveness of the sulfa drugs on races 15B, 17, 38, and 56.

There are differences in effectiveness among these chemically similar compounds. Sulfanilamide inhibits rust development temporarily. After 3 days uredia and rust flecks develop slowly. The final uredial development on plants treated with sulfacetamide, sulfasuxidine, and sulfathiazole approximated that on the control plants, while the rust mycelium did not develop and no pustules were found on plants treated with sulfadiazine, sulfapyrazine, sulfapyridine, and gantrisin. Plants treated with these chemicals showed no visible symptoms of rust infection. A portion of this work on sulfadiazine was reported by Mitchell, Hotson, and Bell (8).

Many compounds were tested by this technique. These chemicals were divided into the following groups: 1) nitrated and hydroxy benzine derivatives, 2) fungicides and insecticides, 3) vitamins, 4) growth substances, 5) antibiotics and 6) a miscellaneous group. (See appendix A). A large number and variety of chemicals were applied by this technique and did not visibly affect development of the rust. In screening tests with these chemicals, sulfadiazine (300 p.p.m.) was included in each experiment as a check on the effectiveness of the spray treatment.

The application rates of the spray were based, for screening purposes, on 300 p.p.m. of the test chemical with 1 per cent Tween-20 added to the solution. Spraying 20 ml. onto 6 pots of wheat, each pot with 10 seedlings, approximated a rate of about 14 lbs. of chemical per acre. Table 2 shows the effect of varying concentrations of the sprays, the concentration at which rust is suppressed without injury to the host, and the dosage at which the host is injured. Sulfadiazine at 800 p.p.m. stunted the wheat plants and caused chlorosis of the leaves, yet plants recovered and in approximately 7 days resumed normal growth. This chemical was effective in suppressing rust at a rate as low as 100 p.p.m., or about 3.5 lbs. per acre.

One notes the range of effective concentration between rust suppression and toxicity to the host. Sulfadiazine, for example, has a range of 200 p.p.m. in which to suppress effectively rust development without host injury. This range of effectiveness is extremely important in seeking a practical chemotherapeutic agent for field use.

T. Johnson's experiments with DDT were repeated several times using race 56 of wheat stem rust and his results were confirmed.

The suppression of rust development induced by the sulfa drugs is that which would be expected if the accepted theory of their action in other organisms is true. Of the theories reviewed by Henry (5) the Wood-Fildes theory of para-amino benzoic acid (PABA) competition has been most generally accepted. The essential metabolite with which the sulfa drugs compete is considered to be para-amino benzoic acid because it will reverse the sulfa inhibition. Since folic acid is also capable of reversing this sulfa inhibition and since the side chain of the folic acid molecule is PABA, it is also thought that PABA is an intermediate in the biological synthesis of folic acid. If this is is true for stem rust, then the addition of PABA should enable the rust to recover.

TABLE 1.—*Effectiveness of sulfa derivatives in suppressing Puccinia graminis tritici Race 15B on four wheat varieties*

Rate: 300 p.p.m. solution plus 1 per cent Tween-20-3.3 ml. per 4-inch pot

Chemical	Infection Type[a]			
	Little Club (Triticum compactum)	Spelmar (T. durum)	Vernal (T. dicoccum)	Khapli (T. dicoccum)
Control	4	4=	4	1=
Control plus Tween-20	4	4=	4	1=
Sulfanilamide	3=	3	3	0;—1=
Sulfamerizine	0;	0;	0;	0;
Sulfadiazine	0	0	0	0
Sulfapyrazine	0—0;	0—0;	0—0;	0—0;
Sulfapyridine	0—0;	0—0;	0—0;	0—0;
Sulfaphridazine	0—0;	0—0;	0	0
Gantrisin	0—0;	0—0;	0—0;	0—0;
2-Sulfanilamido-5 bromopyridine	4	4	4	1=
2-Sulfanilamido-5 chloropyrimidine	4	4	4	1=
2-Sulfanilamido-5 nitrothiazole	4	4	4	1=
2-Sulfanilamido-1,3,4 thiadiazole	0;—3—	3= —4	3= —4	0;
3-Sulfanilamide 6 methyl pyridazine	0	0	0	0
Sulfathiazole	4—	3+	3—	1=
Sulfacetimide	3+	3+	3+	1=
Sulfathalidine	4—	3+	3+	1=
Sulfasuxidine	4—	3+	3+	1=
Sulfamethazine	4—	4—	4—	1=

[a]Infection types after Stakman, Levine and Loegering, Identification of Physiologic Races of *Puccinia graminis tritici*. Paper E-617 Bur. Ent. & Pl. Quarant. (ARA) USDA. 1944.

Tests of this hypothesis showed that 300 p.p.m. of sulfadiazine suppressed the rust, and when this treatment was followed by 300 p.p.m. of PABA the rust resumed growth. Partial resumption of growth of the rust could be obtained when PABA was applied up to 3 days after application of the sulfadiazine; the fourth day only slight recovery occurred. On the fifth day PABA could no longer induce any growth response on the part of the rust. The recovery occurred not only with PABA but also with folic acid. One can therefore conclude that PABA and folic acid are vitamins in the metabolism of wheat stem rust. This conclusion also supports the hypothesis that PABA is a constituent in the biological synthesis of folic acid, which itself may be another essential vitamin for *Puccinia graminis tritici*, as well as for other organisms. A brief report of this was made by Hotson (6).

This recovery is not obtained by adding methionine or glutathione when the sulfadiazine is applied. Conversely, if vitamin K (2-methyl-1, 4-napthoquinone)

TABLE 2.—*Effect of concentration of various test chemotherapeutic agents on rust infection type and wheat toxicity*

Little Club Wheat (*T. compactum*)—Race 56 *Puccinia graminis tritici.*

Seedlings inoculated at 7 days, sprayed at 10 days, readings at 21 days and 25 days. 3.3 ml. per 4-inch pot.

Chemical	Rates in parts per million					
	50	100	200	300	500	800
Control	4	4	4	4	4	4
Control + Tween —20(1%)	4	4	4	4	4	4
Sulfadiazine	3= —4	0;—3=	0—0;	0	0[C]	0[CS]
Sulfapyrazine	4	3=	0;—3=	0	0[C]	0[CS]
Sulfapyridine	4	3=	0;—3=	0	0[C]	0[CS]
Gantrisin	3=	0;—3=	0—0;	0	0[C]	0[CS]
Picric acid	.....	4	0;—4—	0;—3=	0[N]	.....
3,5 Dinitrobenzoic acid	.....	4	0;—4	0;—3=	0[N]	.....
2,4,6 Trinitrobenzoic acid	.....	4	0;—4	0;—4	0;—3=	.....
Hydroquinone	.....	4	3= —4	—[N]	0[N]	.....
8 Hydroxyquinoline sulfate	.....	4	0;—4	—[N]	0[N]	.....
Catechol	.....	4	3= —4	—[N]	0[N]	.....
Amate	.....	4	3—4	0;	0;[N]	.....
Pyridine	.....	4	3= —4	3= —4	0[N]	.....
Saccharin	.....	3—4[CN]	2++	3=4[CN]	4	.....

C—General chlorosis of wheat leaves.
S—General stunting of wheat leaves with subsequent recovery.
N—General necrosis of wheat leaves.

were essential to rust metabolism, the fungicide Phygon (2,3 dichloro-1, 4-napthoquinone) might act as competitive inhibitor. Neither vitamin K nor Phygon XL affected rust development when applied by the technique described above to resistant or susceptible wheat varieties.

SUMMARY AND CONCLUSIONS

Tests demonstrated that certain of the sulfa drugs will control wheat stem rust when applied as a post-infection spray at a rate as low as 5 lbs. per acre (minimum 3.5 lbs. per acre).

This control can be counteracted by para-amino benzoic acid and folic acid, allowing the rust to recover.

These sulfa drugs suppress the development of wheat stem rust and their mode of action is similar to that in other organisms. Para-amino-benzoic acid and folic acid apparently are vitamins in the metabolism of *Puccinia graminis tritici*.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
UNIVERSITY FARM
ST. PAUL 1, MINNESOTA

APPENDIX A

Chemicals tested as therapaeutic agents at 300 p.p.m. Those with (*) were found to have activity and are reported in the body of the article.

Group I. Nitrated and hydroxy benzine derivatives

Dinitro resorcinol	Picric acid (*)
Gallic acid	3,5 dinitrobenzoic acid (*)
Pyrogallol	2,4,6 trinitrobenzoic acid (*)
Salicylic acid	Catechol
Resorcinol	Hydroquinone
Cinnamic acid	8-hydroxyquinoline sulfate
m-dinitro benzene	Tannic acid

Group II. Fungicides and insecticides

Zeorlate	Phenyl mercuric acetate
Mercuric chloride	Chloropicrin
Phenylmercuric trienthenol	Pictol
Triethylene glycol ammonium lacate	Paradichlorobenzine
Teramine	D.D.T. (*)
Phygon XL	Amate (*)

Group III. Vitamins

Vit. K (2 methyl-1,4-naphthoquinone)	Carotene
Alpha trocopherol	Yeast extract
Calciferol	Para-amino-benzoic acid
Vit. A—alcohol	Folic acid
Vit. A—acetate	Ascorbic acid
Vit. A—palmetate	

Group IV. Growth substances

Phenyl hydrazine
Maleic hydrazide
2,4 dichlorophenozyacetic acid
2,4,6 trichlorophenoxyacetic acid
Manganese ethylene bis dithiocarbamate (Courtesy of Dupont)
Isopropyl-N-phenyl carbamate

Group V. Antibiotics (including sulfas)

Penicillin C. (100,000 units)
Ty-Sin. (Gramosidin and Tyrosidin)
Sulfanilamide (*)
Sulfathiazole (*)
Sulfamerizine (*)
Sulfadiazine (*)
Sulfapyridine (*)
Sulfapyrizine (*)
Sulfamethazine
Sulfathalidine
Sulfacetamide
Sulfasuxidine
Gantrisin (*) (3,4 dimethyl-5-sulfanilamido-isolazole)
2-sulfanilamido-5 Bromopyridine
2-sulfanilamido-5 chloropyridine
2-sulfanilamido-5 nitrothiazole
3-sulfanilamido-6 methyl pyridazine (*) (Courtesy of Am. Cyanamid Co.).
2-sulfanilamido-1,3,-4 triadiazole (*)
Sulfaphridazine (*)

Group VI. Miscellaneous compounds

Procaine	Methylene blue (*)
Nupercaine	Sucrose
Aspirin	Glucose
Saccharine (*)	Glucose-1-phosphate
Rustin	

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THE CAUSE OF EPINASTIC SYMPTOMS IN FUSARIUM WILT OF TOMATOES [1]

A. E. Dimond and Paul E. Waggoner

Epinasty is a characteristic early symptom of Fusarium wilt of tomatoes (14). Two compounds of biological origin have been said to cause epinasty: ethylene and ethyl alcohol. In the present paper the production of each by *Fusarium oxysporum* f. *lycopersici* and/or the infected tomato plant is examined, together with the quantities necessary to cause epinasty in tomatoes.

Ethyl alcohol has been considered to be a possible cause of epinasty because tomato cuttings which absorb alcohol at concentrations ranging from 0.5 to 3.0 per cent by volume developed epinastic symptoms (11) and because many *Fusaria* produce alcohol *in vitro*.

Ethylene is known to cause epinasty in tomatoes at concentrations as low as 0.1 p.p.m. in ambient air (1). Ethylene is known to arise either from fungi directly or from injured plant tissue. Ethylene is produced by *Penicillium digitatum* attacking decaying citrus fruits (12) and in culture (16). So far this is the only fungus shown to produce ethylene. Ethylene can also be produced by diseased plant tissue even if the pathogen itself is incapable of forming ethylene. Defoliation that is a normal symptom of such diseases as rose black spot and cherry leaf spot apparently results from the formation of ethylene by diseased tissue (15). Ethylene also arises from plant tissue injured by such phytotoxic compounds as endothal and the ethylene produced in this way has been shown to be responsible for defoliation (6).

A number of analytical methods for ethylene have been used. Being an unsaturated hydrocarbon, it adds bromine to form ethylene dibromide. Consequently the reduction in color of bromine water can be used to detect ethlyene in the presence of ethyl alcohol (13). Ethylene is also oxidized by $KMnO_4$ to form ethylene glycol and the reduction of permanganate can be used as a quantitative measure of ethylene in the absence of other volatile compounds capable of oxidation by permanganate. For example, ethyl alcohol and acetaldehyde could interfere. The latter compounds reduce neutral permanganate slowly whereas the reaction with ethylene is rapid; this property is useful for detecting ethylene in the presence of interfering volatile compounds arising from biological material. Ethylene forms addition compounds with mercuric nitrate (2, 7) or mercuric perchlorate (17). As a result ethylene can be trapped by bubbling a stream of air containing it through a solution of either of these reagents. The ethylene so trapped can be liberated again quantitatively on addition of HCl (2, 7, 17). This is the basis for a manometric method for estimation of ethylene (17).

Methods of bioassay have also been used. Etiolated pea seedlings exhibit the triple response in the presence of as little as 0.2 p.p.m. ethylene (1, 8). The triple response consists of a shortening of the epicotyl, an increase in epicotyl diameter, and a diageotropic curvature of the plumule. The magnitude of these responses is useful for estimating the quantities of ethylene involved.

Ethylene also causes epinasty and tomato plants will detect as little as 0.1 p.p.m. of ethylene in air (1). Although other gases (acetylene, carbon monoxide, propylene, and butylene) will cause an epinastic response, they do not arise from biological processes and are 1000 times less effective than ethylene in causing epinasty (1).

ETHYL ALCOHOL AS A POSSIBLE CAUSE OF EPINASTY IN FUSARIUM WILT.—*Methods.*—In these studies alcohol production was estimated in culture filtrates of *Fusarium* and in the tracheal fluid of healthy and diseased plants. Because small fluid samples were involved in some of these determinations, the refractive index of the distillate of samples was used as a measure of alcohol content when the distillate also tasted of alcohol (9). A modification was made in the standard procedure to increase the sensitivity and adapt it to detecting alcohol in 1 ml. samples of fluid. A 1 ml. sample was delivered from a standardized pipette into a micro-distilling apparatus, and the flask was heated slowly to boiling. The first 3 drops of distillate, being richest in alcohol, were collected on the prism of an immersion refractometer; the auxiliary prism positioned; and the refractive index determined at 25° C. With a standard solution containing 0.963 per cent by volume of ethyl alcohol, the 3 drop sample collected on the refractometer prism contained 2.99 per cent alcohol. In repeated trials the method proved to give an enrichment of alcohol of 3.1 times, thus permitting assay of highly dilute alcohol solution. When alcohol was reported in studies on culture filtrates or tracheal fluids, it was also identified by flavor of the fourth drop of the distillate if possible.

Results.—Ethyl alcohol production by *Fusarium* was determined from 1-month old quiet cultures that had grown on a medium consisting of 0.15 gm. Casamino acids, 1.5 gm. glucose, 0.1 gm. $MgSO_4$, 0.15 gm. KH_2PO_4, 0.1 gm. yeast extract, and 0.1 ml. Hoagland's A-Z mixture of trace elements per 100 ml. The mycelium was removed by filtration and the alcohol content of the filtrate was determined on successive 1 ml. samples. The alcohol content of the culture filtrate proved to be 0.53 per cent by volume and could be smelled and identified by taste. According to Ludwig (11) this amount of alcohol is just sufficient to cause epinasty in tomato cuttings absorbing the solution.

[1] Accepted for publication August 21, 1953.
Research conducted under Contract No. AT-(30-1)-580 between the Atomic Energy Commission and The Connecticut Agricultural Experiment Station.

Tomato plants were likewise inoculated with *Fusarium*. Three weeks later, when wilting and epinastic symptoms were well developed, a number of inoculated and healthy tomato plants were decapitated above the cotyledonary node and 1 ml. of each type of exudate was collected from the bleeding stumps as rapidly as possible. The samples were kept under refrigeration for the 2-hour period over which they were being collected. The alcohol content of the exudate from healthy plants was estimated as 0.08 per cent and from diseased plants as 0.14 per cent by volume. However, no alcohol could be tasted in the samples, and the values given probably represent upper limits because volatile esters that increased the refractive index were distilled over, as was evident from the "mashed plant" flavor of the distillates.

Next the ability of ethyl alcohol to cause epinasty in young tomato plants was examined. Tomato plants supplied with water were placed in a series of empty desiccators where they were exposed to the vapor from aqueous solutions of ethyl alcohol, ranging from 1 to 95 per cent by volume, for a period of 3 days. Alcohol solution remained unevaporated in each desiccator at the termination of the experiment. A control desiccator contained a tomato plant exposed to water vapor. The angle between the petiole and the stem below was measured with a protractor at the beginning and end of the experiment, and the change in angle was used as a measure of epinasty. No epinasty was caused by any of the concentrations of alcohol.

The ability of ethyl alcohol to cause epinasty when it is absorbed as an aqueous solution by the basal end of a tomato cutting was also examined. Cuttings were allowed to absorb solutions ranging from 0.15 to 1.20 per cent by volume for 4 days. In repeated experiments, none of these concentrations produced epinasty. This result was at variance with what has been reported by Ludwig (11). The cause of this difference remains unexplained.

Ludwig has observed that concentrations of ethyl alcohol less than 0.5 per cent do not cause epinasty whereas concentrations ranging from 0.5 to 3.0 per cent do so. To be conservative, his values have been taken as correct for evaluating the possibility that alcohol can be a cause of epinasty in Fusarium wilt. Evidently the concentrations of alcohol produced by culture filtrates could cause epinasty in tomato cuttings which absorb them. However, the amount of ethyl alcohol produced in tracheal fluid of the diseased plant is insufficient to cause epinasty. We conclude that ethyl alcohol does not cause this symptom in diseased plants.

ETHYLENE AS A CAUSE OF EPINASTY IN FUSARIUM WILT.—*Evaluation of the specificity of the triple response for ethylene.*—Evidently ethyl alcohol is produced by *Fusarium* in culture and probably in the diseased plant. Therefore studies on ethylene production by pathogen and infected host must be carefully chosen so that they will distinguish between ethyl alcohol and ethylene. The triple response of peas has been considered specific for ethylene but the effect of ethyl alcohol on etiolated peas apparently has not been examined. This was accordingly done.

A lot of Alaska peas[2] was germinated in 2 ways. One lot (A) was germinated for 2½ days in a rag doll and seedlings were then selected for uniformity and placed on a filter paper in the bottom of each of 4 petri dishes, 15 seedlings per dish. The other lot (B) was soaked overnight in water and 60 seeds were then placed on a sheet of filter paper in the bottom of each of 4 petri dishes. These dishes were then incubated in a dark cupboard for 2 days and seedlings were watered with saturated $CaSO_4$ solution. After 2 days the 15 most uniform seedlings were retained in these dishes. All seedlings were subsequently watered with saturated $CaSO_4$ solution. One petri dish containing seedlings from each lot of seed was placed in each of 4 desiccators (volume 2.1 l.), containing 100 ml. of water or alcohol solution, ranging in concentration from 0.1 to 3.0 per cent. The desiccators were then closed and placed in a dark cabinet at room temperature for 3½ days. The length and diameter of epicotyls and the angle of curvature of the growing point were measured, the lengths converted to logarithms, and the data analyzed.

The results of analyses indicated that ethyl alcohol had significantly changed the 2 most conspicuous criteria in the triple response: logarithm of epicotyl length and angle of the plumule to epicotyl. Diameter of the epicotyl also tended to increase with concentration of alcohol but differences were not significant (Table 1). The concentrations of alcohol used in this study correspond to what can be produced in *Fusarium* cultures. If peas respond in this manner, the triple response cannot be used in a simple way to distinguish between the effects of ethyl alcohol and ethylene.

TABLE 1.—*The response of etiolated peas to alcohol vapor*

Concentration of ethanol	Logarithm of epicotyl length (mm.)		Angle of plumule		Diameter of epicotyl (mm.)	
	Lot A[a]	Lot B[b]	Lot A	Lot B	Lot A	Lot B
0.0%	1.66	1.17	82.3°	99.0°	2.31	2.56
0.1	1.43	0.93	63.0	84.0	2.39	2.56
1.0	0.81	0.61	22.0	67.0	2.66	2.58
3.0	0.89	0.69	10.3	40.3	2.60	2.65
LSMD (P: 0.05)	0.55		62.7°		0.46	

[a] Germinated in rag dolls.
[b] Germinated in petri dishes.

Production of ethylene by Fusarium oxysporum f. *lycopersici.*—Tests for ethylene production by *Fusarium* cultures were made. When the fungus was grown for 3 days on 3 per cent maltose, 0.8 per cent nutrient broth agar, it caused a triple response in peas. Thus in 1 experiment after 11 days exposure to fungus cultures or plates containing sterile medium the average

[2] Kindly supplied for these studies by Associated Seed Growers, New Haven, Connecticut.

length of control pea epicotyls was 6.59 ± 0.35 cm. whereas that of peas exposed to *Fusarium* cultures was 2.88 ± 0.09 cm. Hall (5) has shown in the case of *P. digitatum* that a cell-free extract of mycelium uses several carbohydrates as substrates to produce ethylene, the yield varying with the substrate. When *Fusarium* was grown for 3 days on a 3 per cent carbohydrate-0.5 per cent nutrient broth agar, the magnitude of the triple response after 6 days was greatest when maltose was the substrate, was less strong on pectin, and did not differ from the control response on glucose. These results were confirmed in a duplicate experiment. Thus it was shown that there was a parallel behavior between *Fusarium* and *Penicillium*. Also this evidence pointed to ethylene production rather than ethyl alcohol because the fungus has been shown to produce alcohol on a liquid glucose medium in a 30-day period. Evidently the alcohol produced on a glucose agar medium in 3 days was insufficient to cause a triple response. If so, alcohol would not be produced in 3 days on a pectin or maltose medium either and the triple response can be tentatively assigned to ethylene production.

To identify ethylene more definitely, gas from fungus cultures was studied by chemical methods. In experiments in which the properties of gases over *Fusarium* cultures were studied, 100 ml. of indicator solution, e.g., bromine water, was placed in a 100 ml. gas washing bottle equipped with a sintered glass disc of fine porosity. The output side of the bottle was connected to a flow meter and then through a manifold to an aspirator. The flow meters were used to adust the flow to the same rate through all trains in the system. The input side was connected to a train of fungus cultures, sealed tightly with 2-hole rubber stoppers with glass tubing inserted through these holes but ending above the cultures so as to sweep out air over them. Flasks were connected by rubber tubing and sterile cotton wool was inserted in the tubing between each flask. The control consisted of a number of water-filled flasks and measured the corresponding properties of laboratory air. When cell macerates were employed, a second gas washing bottle containing the macerate was substituted in the system for the train of fungus cultures.

Gases arising from *Fusarium* cultures were passed through bromine water. Three *Fusarium* cultures connected in a train were grown for 8 days in 500 ml. Erlenmeyer flasks on 100 ml. of a casamino acids nutrient of the composition used in the alcohol studies except that maltose was substituted for glucose. The bromine water was prepared by diluting 141 ml. of a refrigerated stock solution to 1 liter, the stock solution containing 20 gm. KBr and 2.8 ml. bromine per liter. Small amounts of bromine are lost from this solution when air is bubbled through it and a slight loss in color of control solutions is to be expected. The change in opacity (logarithm of per cent absorption) of the bromine water was measured in a colorimeter, using a blue (425 mmu) filter. In a 30-minute bubbling period, laboratory air changed the opacity of the bromine water 6.3 units whereas gases arising from *Fusarium* cultures produced a change of 33.5 units. This result was repeated in independent experiments. Evidently a volatile compound arising from *Fusarium* cultures reacts with bromine, a characteristic of acetaldehyde and unsaturated hydrocarbons but not of ethanol (13).

To determine whether acetaldehyde was produced by *Fusarium* cultures, gas arising from them was passed through the fuchsin-aldehyde reagent which is highly sensitive to aldehydes (13). The reagent was prepared by adding 0.5 gm. fuchsin to 500 cc. distilled water, filtering, decolorizing with SO_2, and allowing the solution to stand overnight before use. The fuchsin-aldehyde reagent was placed in the gas washing bottles. In this experiment 3 trains were used: 1) the train of fungus cultures, 2) the control train, and 3) a train in which laboratory air was passed over a 0.2 per cent solution of acetaldehyde. Gas was passed at the same rate through the 3 trains for 80 minutes. Then the opacity of the 3 solutions of fuchsin-aldehyde reagent was determined with a colorimeter, using a green (525 mmu) filter. The change in opacity of the fuchsin-aldehyde reagent was 0.0 in the train of fungus cultures, 0.0 in the control train and 31.0 in the train receiving air passing over 0.2 per cent acetaldehyde. Evidently the amount of acetaldehyde produced by the fungus cultures was negligible and bromine water was being decolorized by an unsaturated hydrocarbon. Ethylene is the only unsaturated hydrocarbon known to be produced by plant tissue (1).

Unsaturated compounds decolorize aqueous solutions of potassium permanganate, the rate of color loss depending on solubility (13). Ethylene is soluble in water to the extent of 12 cc. per 100 ml. at 20° C. and the quantities involved in these studies would be completely dissolved in an efficient gas-washing cylinder. $KMnO_4$ solutions should be decolorized rapidly if appreciable amounts of ethylene are formed by *Fusarium*.

In these studies a dilute $KMnO_4$ solution (0.0012 N) was used and the gain in opacity to blue light (425 mmu) was measured colorimetrically. To determine whether the change in opacity of the permanganate solution was proportional to the amount of oxidizable material present, varying amounts of ethyl alcohol, ranging from 0.2 to 7.0 mg., were added to 20 ml. of $KMnO_4$ solution so that the final volume of the mixture was 21 ml. Over this range, the curve relating opacity of the solution to the amount of oxidizable material added was linear, the slope of the curve increasing with time for at least 24 hours. Because ethylene reacts rapidly with permanganate whereas ethyl alcohol reacts slowly, the change in color of permanganate solutions exposed to gases arising from fungus cultures will be almost wholly a result of oxidation of ethylene if readings are made after very short exposures of permanganate.

The effect of gases arising from *Fusarium* cells and from cell macerates on the permanganate indicated

was determined. Fifteen day old mycelial mats of *Fusarium* grown on a casamino acid-maltose nutrient were recovered by filtration, washed and divided in 2 lots. One lot was resuspended in 100 ml. of citrate-phosphate buffer (pH 6.25) containing 2 per cent maltose, and macerated for 10 minutes in a Waring blendor. The other lot was suspended in the maltose-buffer without maceration. These and 100 ml. of maltose-buffer were placed in 3 gas washing cylinders which were in turn connected to gas washers containing permanganate. The change in opacity of the permanganate to blue light (425 mmu) was determined after 2 and 4 hours of bubbling. The change in the control train was subtracted from that of the test solutions to correct for the oxidizable components in laboratory air.

This experiment indicated that an oxidizable material (ethylene) was produced by mycelium and by macerated cells (Table 2). Although macerated cells produced less ethylene than mycelial mats, ethylene was produced by macerated cells in measurable quantities. This suggests that an enzyme is responsible for ethylene production, as Hall has found for *P. digitatum* (5).

TABLE 2.—*Reduction of $KMnO_4$ by ethylene produced by mycelial mats or macerate of Fusarium suspended in buffer-maltose solution (pH 6.25)*

Time of aeration hours	Change in opacity of $KMnO_4$	
	Mycelium	Macerate
2	15.6	3.9
4	59.2	11.7

Next the heat stability of the active material in macerated cells was examined. Mycelial mats from 8 day quiet cultures were washed and then macerated in a small volume of citrate-phosphate buffer (pH 6.25) in a Waring blendor for 10 minutes. Half of the resulting macerate was retained for further use and the other half was autoclaved at 15 lbs. for 20 minutes. Each preparation was then suspended in a total volume of 200 ml. buffer. One half of each preparation (100 ml.) was used directly and 2 gm. maltose was added to the other half. These 4 suspensions were then placed in separate gas washing cylinders which were in turn connected to bubblers containing permanganate. Control bubblers contained buffer only or 2 per cent maltose buffer. Air was passed at the same rate through the 6 trains overnight. Then the change in opacity of permanganate caused by test solutions was corrected for the oxidizable components of laboratory air. The production of ethylene by autoclaved macerates was negligible whereas unheated macerates produced measurable amounts (Table 3). Greater amounts were apparently produced when maltose-citrate buffer was the substrate than when citrate buffer alone was available. These results confirm the conclusion previously drawn. A heat labile enzyme is separable from *Fusarium* cells which uses maltose or citrate as a substrate to produce ethylene.

TABLE 3.—*Loss of ability of Fusarium macerates to produce ethylene on autoclaving*

	Change in opacity of $KMnO_4$ solution	
	Buffer only	Buffer-maltose
Macerated cells	44.5	71.0
Autoclaved, macerated cells	3.0	6.0

Two other methods were used for separating the ethylene-producing enzyme from *Fusarium* cells. Mycelial mats were removed from 14-day quiet cultures of *Fusarium* and divided into 2 lots. The mats of one lot (A) were resuspended on fresh ¼ strength Czapek's solution overnight, and the nutrient filtered, and the mycelium discarded. One hundred ml. of the filtrate, to which 2 gm. of maltose were added, was placed in a gas washing cylinder ahead of one containing 100 ml. of permanganate. The other lot (B) of mycelium was extracted by Hall's procedure (5). Mycelium was washed and then macerated in a Waring blendor for 10 minutes in 100 ml. of 10 per cent aqueous acetone. The resulting macerate was then filtered on a Buchner funnel, the filtrate treated with neutral lead acetate and finally with potassium oxalate to remove the lead. It was then centrifuged and $CaCl_2$ added until oxalate was removed. Finally the liquid was centrifuged again, the supernatant made to 100 ml. volume with water, and 2 gm. maltose added. This was placed in another gas washing cylinder ahead of a second one containing permanganate. The control train of 2 gas washing cylinders contained 2 per cent maltose and permanganate indicator, respectively. Air was passed through this system for 36 hours and then the change in opacity of the permanganate was measured as previously described. The change in opacity of permanganate exposed to extract A was 47.0 units whereas the indicator exposed to extract B changed in opacity by 29.0 units after the change in control indicator was deducted. Evidently the enzyme can be extracellular because it was found in nutrient media on which mycelium was suspended and then removed. This method resulted in more ethylene production than maceration of cells followed by precipitation of component salts and cell fragments.

Finally mercuric perchlorate reagent was used to identify ethylene as the product arising from *Fusarium* cells. Ethylene, trapped in mercuric perchlorate, has been estimated manometrically by liberating it again with HCl. Amounts as small as 0.2 ml. ethylene can be detected by this method (17). It has been used to determine ethylene production by *Penicillium digitatum* (16).

Mercuric perchlorate solution was prepared (17). A train of 30 cultures of *Fusarium* was grown on casamino acids-maltose nutrient so that air could be passed through the train into a gas washing cylinder containing the mercuric perchlorate reagent. The amount of ethylene produced by these cultures in 24 hours could not be detected in several trials using the manometric procedure (17). However, the method itself was

shown to be satisfactory by trapping small amounts of ethylene and other olefins from illuminating gas and liberating them with HCl. Therefore it was concluded that either the amount of ethylene produced by *Fusarium* was considerably less than is formed by *Penicillium digitatum* or that the efficiency of the gas washing cylinder used for trapping ethylene in these studies was very low as compared with the apparatus used by Young, et al (17).

A more sensitive measure for ethylene was evidently necessary. Ethylene will cause epinasty in a tomato plant at concentrations of 0.1 p.p.m. (1). A young tomato plant should show epinasty in a 2.1 l. closed vessel if as little as 0.21 mm^3. of ethylene is present. This method should therefore be about 1000 times as sensitive as the manometric method.

Several small tomato plants were placed in beakers of water, the angles between the petiole and the stem below measured for the third and fourth leaves above the cotyledonary nodes, and 2 plants placed in each of two 2.1 l. vacuum desiccators. Each desiccator was evacuated to a pressure of 17 mm. Hg and then closed. The vacuum in the control desiccator was slowly released and it was allowed to stand for 2 days in the laboratory. The second desiccator was attached to the fungus cultures and the vacuum slowly released. This drew the gases above the fungus culture into the desiccator and around the test plants. The combined volume of air over the fungus cultures was greater than that of the desiccator so that no outside air was drawn into it when vacuum was released. Two days later the angles of the petioles on check and treated plants were again measured. The average change in angle of 8 petioles on check plants was 1° whereas on the 8 petioles of plants exposed to the gases arising from fungus cultures it was 50°. This difference was significant at the 5 per cent level. The effect must have been caused by ethylene, since we have already seen that tomato plants exposed to alcohol vapor did not show epinasty.

In a second experiment the perchlorate method was used to identify the gas as ethylene. Vacuum desiccators were employed in the same manner as previously described except that a petri dish containing 10 ml. of mercuric perchlorate reagent was placed in the bottom of each. As before, 1 desiccator was connected to the train of cultures after it had been evacuated and the vacuum was then slowly released. The vacuum in the second desiccator was slowly released so that laboratory air was drawn in. This process was repeated twice in 1 day. Then 2 tomato plants were placed in each desiccator after petiole angles had been measured. A small vial containing 4 equivalents of 2 N HCl per equivalent of mercury in the perchlorate reagent was also inserted. After the desiccators had been closed, the HCl was tipped into the perchlorate reagent. The next morning there was a severe epinastic response on the plant exposed to perchlorate reagent exposed to gases arising from fungus cultures, whereas on control plants, it was only slight. Thus the average change in petiole angle on control plants was about 35° whereas on plants exposed to material arising from fungus cultures and trapped by mercuric perchlorate the change was 165°. This difference was also significant.

This specific test for ethylene confirms that previously presented: ethylene is a metabolic product of *Fusarium* cells. It seems likely that this fungus produces less ethylene than does *Penicillium digitatum*, but it appears to produce considerable amounts nonetheless.

Production of ethylene by Fusarium infected tomato plants.—The production of ethylene by diseased tomato plants was examined. Tomato plants were inoculated with *Fusarium oxysporum* f. *lycopersici* or were left uninoculated. When symptoms in inoculated plants were severe, a number of diseased plants, weighing 9 gm. in all, were placed in an empty desiccator with etiolated pea seedlings while healthy plants weighing 9 gm. were placed in a second desiccator of the same volume together with other pea seedlings. After exposure for 3 days the magnitude of the triple response was compared in the 2 cases. Some active gas was produced by healthy plants, as was evident from the diageotropic curvature of the pea epicotyls. Considerably more active material was produced by the diseased plant because the peas showed a more pronounced diageotropism and also epicotyls were of larger diameter and were shorter. Evidently a diseased plant liberates a gas which is capable of producing the triple response in peas whereas a healthy plant produces much less of this material.

As we have seen, however, the triple response is not reliable for distinguishing between ethylene and ethyl alcohol, inasmuch as alcohol produces a triple response from solutions no more concentrated than occur in diseased plants.

However, a healthy tomato plant responds epinastically to ethylene but not to ethyl alcohol when these materials are present at low concentrations in the atmosphere. Therefore, healthy 6 week old Bonny Best tomato plants were placed in a closed container for 4 days with 2 other tomato plants which had been inoculated with *Fusarium* 2 weeks previously and had severe disease symptoms. As controls, other indicator plants were confined for the same time with healthy tomato plants of the same age as the diseased ones. At the end of the experiment angles between petioles and the stem below were measured for all except the 2 bottom leaves. The accumulated angles at which petioles emerged from the stem were 420° for indicator plants enclosed with diseased tomatoes and 670° for control plants enclosed with healthy tomatoes. The difference between these, 250°, was greater than the least significant difference, 243°, at the 5 per cent level when data were analyzed by the method of Link and Wallace (10). In a second experiment these results were confirmed, also with a significant difference. These results suggest that ethylene is produced by the fungus and by the diseased host and is responsi-

ble for the epinastic symptoms of Fusarium wilt of tomato.

RELATION OF ETHYLENE PRODUCTION TO OTHER SYMPTOMS OF FUSARIUM WILT.—Defoliation is another symptom of Fusarium wilt. Williamson has demonstrated the role of ethylene in producing defoliation in a number of plant diseases (15). The effect of ethylene in causing defoliation of tomato plants was examined. Healthy tomato plants were repeatedly exposed to gaseous ethylene. Whereas they showed pronounced epinasty promptly and a slow yellowing of older leaves, these leaves did not abscind.

Next the possibility was examined that ethylene, absorbed in aqueous solution by the plant, might produce these effects when ethylene absorbed as a gas would not. Ethylene was trapped from illuminating gas by mercuric perchlorate solution and was liberated again by adding HCl. As ethylene was liberated, it was bubbled through distilled water and a solution approaching saturation (12 cc/100 ml. water at 20°C.) was eventually obtained. Tomato plants were uprooted, washed, and allowed to stand in this solution. Severe epinasty promptly appeared. The solution in which plants were standing was replaced with fresh ethylene solution 3 times in a 10-day period. Epinastic symptoms persisted and yellowing of the oldest leaves gradually followed. However, no defoliation occurred as a result of this treatment. Control plants in water for the same period developed neither epinastic symptoms nor yellowing of oldest leaves.

Because tomato plants infected with *Fusarium* become drought hardened as symptoms develop (3), it is possible that a drought-hardened plant will become defoliated on exposure to ethylene when a succulent tomato plant will not. Therefore drought-hardened tomato plants were exposed to ethylene both as a gas and in water solution in the manner already described. Drought-hardened plants not exposed to ethylene served as controls. These plants finally responded as succulent plants had; epinasty developed along with yellowing of old leaves but there was no defoliation. Accordingly it is concluded that although ethylene is responsible for epinastic symptoms in Fusarium wilt, it is not responsible for the defoliation which is a normal disease symptom.

Ethylene is known to stimulate the development of root initials in many plants and the effect is particularly striking on the tomato. Both Wellman (14) and Fisher (4) have noted as an occasional symptom the stimulation of root initials in tomato plants infected with *Fusarium*. It is not unlikely that this effect is caused by ethylene.

SUMMARY

Ethyl alcohol and ethylene have both been considered to be possible causes of epinasty, an early symptom of Fusarium wilt of tomatoes. Ethyl alcohol is produced by *Fusarium oxysporum* f. *lycopersici* in culture and by the pathogen and/or the diseased host. However, the amounts involved are insufficient to cause epinasty in healthy tomatoes, either when absorbed as solutions by cuttings or as vapor through leaves.

Ethylene is produced by *Fusarium* in culture and in the diseased host. In culture, ethylene production by *Fusarium* was demonstrated by its ability to cause the triple response in peas, epinasty in tomatoes, and by such chemical properties as bromine addition, permanganate reduction and reversible trapping by mercuric perchlorate. Further, ethylene production varied with the substrate, was stopped when cells were autoclaved and could be obtained with cell-free extracts on a suitable substrate. *Fusarium oxysporum* f. *lycopersici* is therefore the second plant pathogen found to contain this enzyme system, *Penicillium digitatum* being the first (5, 16). Ethylene production by infected tomato plants was also demonstrated by producing greater amounts of epinasty in healthy indicator plants confined with diseased ones than noninoculated plants would produce. Also diseased plants caused a triple response in peas. Thus ethylene production is responsible for the epinastic symptoms of *Fusarium* wilt of tomatoes. It also appears responsible for the production of root initials, occasionally stimulated to develop on infected plants. But ethylene production is not responsible for defoliation of *Fusarium* infected tomatoes, a conspicuous disease symptom. This was demonstrated by exposure of tomato plants to ethylene gas or to aqueous solutions which plants absorbed.

Finally, the triple response of peas was shown to be caused not only by ethylene but by ethyl alcohol vapor as well. It is therefore unreliable as a method for identifying ethylene.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

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THE RELATION OF HOST NUTRITION TO MULTIPLICATION OF TURNIP VIRUS 1 IN NICOTIANA GLUTINOSA AND N. MULTIVALVIS [1]

Glenn S. Pound and Lewis G. Weathers

In an earlier paper reporting the effects of host nutrition on the concentration of cucumber virus 1 in spinach (2) it was pointed out that published data in this field have been contradictory. Most published data have dealt with the effects of nutrition on multiplication of tobacco mosaic virus in tobacco, and especially the relationship of nitrogen nutrition to multiplication. This paper reports the effects of various nutrients and nutritional levels on the multiplication of turnip virus 1 in *Nicotiana glutinosa* L. and *N. multivalvis* Lindl. A preliminary report has already been made (14).

Materials and methods.—Seed of *N. glutinosa* and *N. multivalvis* was sown in white silica sand to which had been added a very dilute balanced nutrient solution. Subsequent waterings were with distilled water. A few weeks later seedlings of uniform size were transplanted into silica sand in crocks equipped with a siphon similar to that described by Pryor (8). The siphons automatically drained off excess nutrients and kept the sand at the same moisture level in each crock. After transplanting, each crock received, at weekly intervals, a prescribed amount of nutrient solution under study. The solutions used were identical with those described by Cheo et al. (2). When the plants had shown a differential growth response to the treatments they were inoculated with the cabbage A (5, 13) strain of turnip virus 1, care being taken that all plants were comparably inoculated. There were usually 3 or 4 crocks of 6 plants each for each treatment. One of these was noninoculated and was used to measure effects on growth.

Virus concentration was measured by half-leaf inoculations to *N. tabacum* L. var. Connecticut Havana 38 on which the virus produces only necrotic local lesions. Finely powdered carborundum was applied as evenly as possible before inoculation with a powder blower attached to a compressed air line. Four nutrient treatments were used in each experiment and treatment comparisons were made in all possible combinations in the incomplete block design described by Pound (6).

In preparing the inoculum for assay, a composite sample of 1 gm. of tissue from all the plants in a given treatment was macerated in a given amount of distilled water until the mixture was homogeneous, in 10 ml. of water for tissue from *N. glutinosa* and in 30 ml. for tissue from *N. multivalvis*. In most experiments, the youngest unfolded tip leaves were assayed, but some assays were of inoculated leaves or of the entire plant. When they were of the entire plant liquid aliquots of expressed juice were sampled.

Greenhouse air temperatures were maintained at a level favorable for virus multiplication in the specific host in question (7). Tests of significance between comparisons of any 2 treatments for any 1 assay were calculated by analysis of variance. The treatment average of the 3 or 4 assays of a given experiment were determined. These were then averaged to obtain the treatment average for all experiments. For simplification, therefore, the data presented for given treatments are the overall averages of all experiments. These treatment averages do not always accurately indicate the difference between treatments due to variations between leaves of the assay plant so supplementary statements regarding significance are made in the text.

Experimental results.—*Nitrogen.*—Four experiments with *N. glutinosa* and 3 with *N. multivalvis* were made. In the first experiment with *N. glutinosa* 6 levels of nitrogen (21, 70, 210, 420, 630 and 1050 p.p.m.) were used. Growth response to the various nitrogen levels showed a gradual increase in both green and dry weight up to 630 p.p.m. Above this level growth decreased. It was found that 21, 210, 530 and 1050 p.p.m. of nitrogen gave a representative growth curve and in all subsequent trials 70 and 420 p.p.m. solutions were omitted.

Both hosts showed the same growth response to the different nitrogen levels. Healthy plants growing at 21 p.p.m. showed symptoms of severe nitrogen de-

[1] Accepted for publication August 21, 1953.
Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation.
Present address of junior author is Citrus Experiment Station, Riverside, California.

ficiency. They were markedly stunted and chlorotic and grew very little over the entire length of the experiment. In all experiments the maximum growth was at 630 p.p.m. Plants at 1050 p.p.m. showed effects of excess nitrogen; leaves were very dark green and plants were stunted. Growth response of diseased plants at the various nitrogen levels was similar to that of healthy plants.

Symptoms appeared at approximately the same time in all groups of plants. Ultimate symptom expression, however, varied with the treatment. A slight gradient of symptom severity existed, increasing from the low nitrogen up to 630 p.p.m. At 1050 p.p.m. symptoms were very mild and remained so throughout the experiment.

Results of the virus concentration assays are shown in Fig. 1. Tests were started when systemic symptoms first appeared and subsequent assays were made at various intervals thereafter. In 1 experiment with *N. glutinosa* inocula for the first assay were taken from inoculated leaves prior to the appearance of systemic symptoms and in the final assay whole plants were harvested. With both hosts and in all assays virus concentration coincided closely with vegetative growth and symptom severity and not with the amount of nitrogen supplied. Plants low in nitrogen and those with excess nitrogen had a low concentration of virus. At 630 p.p.m., where maximum growth occurred, virus production was highest. The F values calculated for the various treatment comparisons of 12 assays of *N. glutinosa* showed that significant differences occurred in all cases except in occasional assays in which no differences occurred between the lowest and highest levels and in 1 assay in which there was no difference between the 210 and 630 p.p.m. levels. Of 9 assays with *N. multivalvis* significant differences occurred in all comparisons except between 21 and 1050 p.p.m. in 3 assays and between 210 and 630 p.p.m. in 4 assays.

It is well to note that in *N. glutinosa* the correlation between host growth and virus concentration was evident from the time of the assay from inoculated leaves through all subsequent assays. This would indicate that virus multiplication throughout the disease cycle was favored by nitrogen nutrition levels that enhanced host growth. With *N. multivalvis* there was some indication, however, that the rate of virus multiplication was slower at the highest nitrogen level than at the low nitrogen levels. Lesion counts of plants grown at 21 p.p.m. at the first assay were not significantly different from those at 1050 p.p.m. In later assays the virus concentration of plants at 1050 p.p.m. increased in comparison to other treatments. At the final assays lesion counts at the highest level were significantly higher than counts at 21 p.p.m. and in 1 experiment they were significantly higher than 210 p.p.m. Apparently, virus multiplication at 1050 p.p.m. was retarded, but over a given length of time the total virus concentration became as high or even higher than virus concentration of the nitrogen-starved plants.

Phosphorus.—Four levels of phosphorus (3, 93, 237, and 547 p.p.m.) were used in 3 separate experiments with each host. The effects of varying amounts of phosphorus on host growth and virus production are shown in Fig. 1. In each experiment with each host the growth curve of healthy plants was similar. Growth responses at the various phosphorus levels were similar in both diseased and healthy plants. The best growth as measured by fresh and dry weights occurred at 237 p.p.m. Plants grown at 547 p.p.m. were stunted. At 3 p.p.m. the plants were markedly stunted and dark green in color.

Virus symptoms appeared at approximately the same time in each treatment. Differences in the progression of symptoms and ultimate symptom severity could not be distinguished among the different treatments. At the time of the final assay all the treatments showed severe symptoms.

It can be seen from Fig. 1 that in both hosts virus concentration increased as the phosphorus supply increased. Analysis of variance showed that in all assays differences between all treatments were highly significant. The least amounts of virus occurred at the lowest phosphorus levels and the greatest amounts occurred at 547 p.p.m. even though plants at this level were retarded in growth. In all assays plants growing at the higher levels of phosphorus showed a much higher virus concentration than plants at the lower levels.

In 1 experiment with *N. multivalvis* the first assay was made from inoculated leaves prior to the appearance of the first systemic symptoms. Highest virus concentration in this assay was at the highest phosphorus level. This was also found to be the case in subsequent systemic assays. This would mean that virus concentration from the time of introduction of the virus was higher at high phosphorus levels than at low phosphorus levels and remained so throughout the disease cycle. Assays of inocula taken from whole plants gave the same concentration curve.

Potassium.—Plants were grown at 8, 78, 704 and 1408 p.p.m. of potassium in otherwise identical nutrient solutions. There were 3 experiments with *N. glutinosa* and 2 with *N. multivalvis*. Both hosts showed identical growth responses to the various treatments. The low-potassium plants (8 p.p.m.) showed typical symptoms of potassium deficiency whereas high-potassium plants showed an equal retardation of growth but no other effects. Plants growing at 78 and 704 p.p.m. showed little difference in growth but growth at either level was considerably greater than that at the low or excess potassium solutions. Symptoms of systemic infections in potassium-starved plants usually appeared 1 or 2 days earlier than in plants receiving ample potassium. There was no distinguishable difference in ultimate symptom severity among the various groups of plants.

With *N. glutinosa*, growth was greatest in 1 experiment at 78 p.p.m. and in 2 experiments at 704 p.p.m. With *N. multivalvis* in each experiment growth at 78

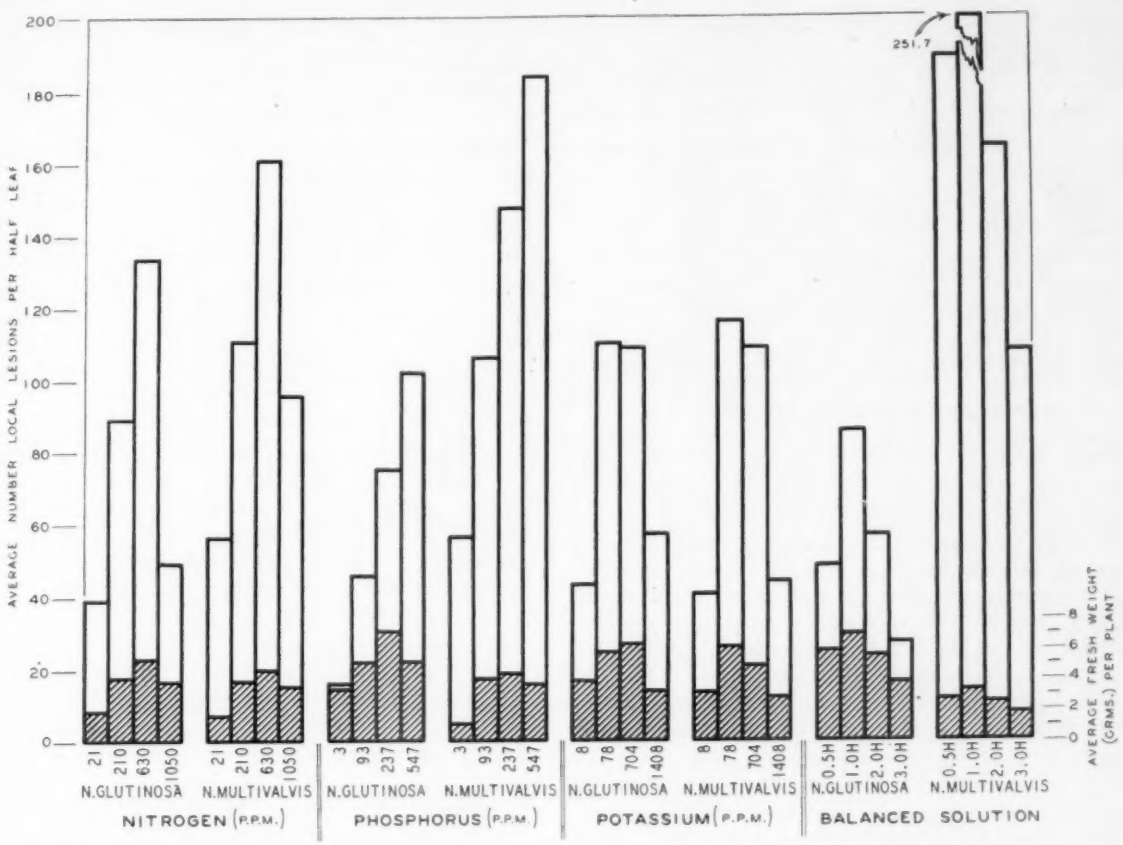

FIG. 1. The relation of host nutrition to the concentration of turnip virus 1 in *N. glutinosa* and *N. multivalvis* as measured by infectivity of sap extracts.

p.p.m. was slightly greater than at 704 p.p.m. In all cases the highest virus concentration occurred at the level giving the greatest growth. However, the differences between the 78 and 704 p.p.m. levels with either host were not significant in regard to either growth or virus concentration. Likewise, there were no significant differences in the effects of the 8 and 1408 p.p.m. levels on virus concentration or host growth. Virus concentration and host growth at either of the 2 intermediate potassium levels, however were significantly higher than corresponding values at either the lowest or highest levels (Fig. 1).

Concentration of Balanced Solutions.—Plants were grown in varying concentrations of a balanced solution, the basic solution being designated as 1H and being the one described by Hoagland and Snyder (4). In these experiments the basal solution was reduced to one-half (0.5H), increased twice (2H) and 3 times (3 H). Thus, the concentration of nutrients at 3H was 6 times that at 0.5H. Each solution received the same amount of trace-element solution and each solution was adjusted to pH 6.0–7.0. There were 3 experiments with *N. glutinosa* and 2 with *N. multivalvis*.

In each experiment and with each host maximum growth developed at the 1H level with marked stunting at the higher nutrient levels. The results (Fig. 1) reveal that in general there was a positive correlation between host growth and virus activity. Assays of inoculated leaves at 5–8 days after inoculation showed that host growth and virus activity closely paralleled each other. The most lesions were obtained from plants grown at 1H and the fewest lesions from plants at 3H. With *N. glutinosa* at 20 days after inoculation extracts of plants at the 2H level had more virus even though maximum growth was recorded at 1H. There was a suggestion that virus multiplication at the higher nutrient levels was retarded. Ultimate virus concentrations at the 2H level, over a given period of time, exceeded the virus concentration at the 0.5H and 1H levels where growth was at a maximum. F values calculated for the various treatment comparisons in *N. glutinosa* showed that significant differences occurred between all comparisons in 10 assays except between 0.5H and 2H in 4 assays; between 0.5H and 3H in 1 assay; between 1H and 2H in 1 assay, and between 0.5H and 3H in 1 assay.

In general, symptom severity coincided directly with virus concentration but at the time of the final assays there was little difference in symptom expression at 0.5H, 1H and 2H levels. Plants were not producing any new growth and most of the lower leaves were dead. Plants at the 3H level made very little growth

TABLE 1.—*The effect of increasing nitrogen and phosphorus concurrently on host growth and multiplication of turnip virus 1 in Nicotiana glutinosa*

Nitrogen and phosphorus levels (p.p.m.)	Average number of local lesions per half leaf at indicated days after inoculation of *N. glutinosa* plants [a]			Average weight of healthy plants (gm.)	
				Green	Dry
Experiment 1	10 days	15 days	19 days		
21 N, 3 P	21.3	48.8	51.1	6.29	0.71
210 N, 31 P	72.7	93.7	79.7	11.43	1.24
630 N, 237 P	102.7	96.2	90.1	13.77	1.44
1050 N, 547 P	33.6	46.7	49.3	8.44	0.90
Experiment 2	12 days	21 days	----		
21 N, 3 P	120.8	56.0	----	4.41	0.45
210 N, 31 P	177.4	76.8	----	9.68	1.02
630 N, 237 P	184.9	64.9	----	9.38	1.27
1050 N, 547 P	90.8	37.3	----	6.50	0.80

[a] Values shown are averages of 9 replications.

throughout the experiment due to the high concentration of the solution. Symptoms at 3H never reached the severity of symptoms of the other levels.

With *N. multivalvis* analysis showed that the lesion count at 3H was always significantly lower than for any other treatment and 1H was always significantly higher. Significance was shown in comparisons between 0.5H and 2H in only 3 of 6 assays. A decreasing gradient of symptom severity between 1H and 3H closely approximated the virus concentration curve. The correlation of host growth and virus concentration was evident in all assays, indicating that the virus concentration from the time of introduction of the virus into the host was higher at the optimal growth levels. There was no indication that virus multiplication in plants growing at the higher concentrations was in any way retarded as was found with *N. glutinosa* since identical virus concentration curves occurred in all assays.

Increasing nitrogen and phosphorus concurrently.—In the nitrogen experiments it was found that virus concentration coincided with plant growth. At nitrogen levels sufficient to induce stunting virus concentration was reduced. With phosphorus it was shown that virus concentration paralleled the amount of phosphorus supplied to the host even though growth at the highest level was reduced. In view of the results with nitrogen and phosphorus, 2 experiments with *N. glutinosa* were conducted in which nitrogen and phosphorus were increased concurrently starting at the level where both elements were deficient and extending to the level where both elements were in excess.

Growth response to the various treatments (Table 1) showed a marked decrease in both green and dry weights at the lowest and highest nutrient levels. Growth at the 2 intermediate levels was very similar and much greater than at either the low or high level. Plants growing at the 21 p.p.m. nitrogen and 3 p.p.m. phosphorus level were chlorotic and stunted and made little growth throughout the experiments. Plants at the 1050 p.p.m. nitrogen and 547 p.p.m. phosphorus level showed the effects of the excess nutrients; leaves were very dark green and the plants were stunted. Growth response to the various nutrient solutions in diseased plants was similar to that in healthy plants.

Symptoms appeared at approximately the same time in all groups of plants. Severity of symptoms, however, varied with the treatment. The most severe symptoms occurred at the 2 intermediate levels. Symptoms at the lowest and highest nutrient levels were very mild throughout the experiment.

It can be seen from lesion counts of the various assays of the 2 experiments (Table 1) that virus concentration was directly correlated with the growth curve of the healthy plants, as was the case when only nitrogen was varied, and not with the amount of nutrients supplied to the host, as was the case with phosphorus. The presence of the excess phosphorus at the excess nitrogen level did not alter the effect of the nitrogen. In other words the inhibitory effect of excess nitrogen on virus multiplication when in combination with excess phosphorus modified the stimulatory effect of the extra phosphorus on virus multiplication.

DISCUSSION.—Published works on the effect of host nutrition on virus multiplication in plants have largely been restricted to how changes in the supply of nitrogen affect the activity of tobacco mosaic virus in expressed saps. Studies by Bawden and Kassanis (1) reveal that other nutrients also affect the activity of tobacco mosaic virus. Evidence presented in this paper and recent work by Cheo et al. (2) show that the activities of other viruses are materially affected by changes in host nutrition.

Since virus multiplication is confined to the living cell, it would be expected that any interruption in the supply of available nutrients within the cell, and changes in the cell metabolism due to variations in nutrients supplied to the host plant, would materially affect virus activity. Virus activity is probably affected by changes in host nutrition in 2 general ways. One is a direct limitation of nutrient substrate necessary for synthesis of the virus protein. Secondly, nutrient variation might indirectly alter virus concentration by disrupting certain metabolic functions necessary for virus synthesis. The marked correlation between host growth and virus concentration as reported herein would indicate that this latter possibility may be the

more important. Variations of nutrient elements that do not become part of the virus protein should only affect virus activity indirectly by upsetting vital metabolic processes, whereas variations in the supply of those nutrient elements that serve as building material for the virus protein would be expected to affect virus activity both directly and indirectly.

In this study, using a local lesion reaction on tobacco to determine the relative virus activity of expressed sap from *N. glutinosa* and *N. multivalvis* plants infected with turnip virus 1, it was found that with the exception of phosphorus, the effects of the various nutrients on virus activity mainly reflected responses in plant growth. Nutrients that stimulated plant vigor also increased virus production and vice versa. This correlation of plant growth and virus concentration was evident in all assays irrespective of whether the inocula were taken from inoculated leaves, from systemically-infected tip leaves, or from whole plants. With phosphorus it was found that virus multiplication coincided with the amounts of phosphorus supplied to the host even though growth was retarded at the higher levels.

The results of these studies concerning the effect of nutrition on the host-virus relationship show many points of similarity with other workers and also many points at variance. Recent work (2) with cucumber virus 1 in spinach shows almost identical results with data reported in this paper. In that study concentration of the virus increased with increasing growth of spinach up to 630 p.p.m. of nitrogen beyond which there was a decrease in both growth and virus concentration. Further similarity was shown in the case of phosphorus where virus assays indicated that phosphorus levels high enough to effect stunting did not retard virus multiplication but increased it. In the cases of potassium and the concentration of balanced solutions virus concentration appeared to be directly related to growth.

Spencer (9) on the other hand reported that the concentration of tobacco mosaic virus in tobacco was directly related to the amount of nitrogen supplied to the host and was independent of the effect on host growth. Local lesion counts indicated that virus concentration was greatly reduced in the expressed juice of nitrogen-deficient plants as compared to juice receiving ample nitrogen. The highest concentration of virus was from plants receiving excess nitrogen even though plant growth was inhibited. He concluded from later studies (10, 11) that the increased virus activity associated with an increased nitrogen supply was due to an increase in the rate of virus multiplication whereas the decrease in virus activity at low nitrogen levels was due to a limitation in the supply of nitrogen necessary for multiplication. He further stated (12) that the interruption of the nitrogen supply to systemically-infected plants not only inhibited further virus multiplication but also inactivated the virus already present which led to a reduction in infectivity. Chessin (3) also showed a reduced infectivity of tobacco virus 1 in nitrogen-deficient tobacco plants when infectivity was measured on Golden Cluster bean but not when *N. glutinosa* was used as the assay host.

Bawden and Kassanis (1) reported that additions of nitrogen to plants grown in soil did not increase virus concentration or plant growth unless phosphorus was also added. However, with plants grown in sand their data indicated that increases in nitrogen markedly affected host growth and also affected virus concentration without the increased phosphorus. It was also shown in sand cultures that nitrogen levels high enough to cause stunting in some cases even increased virus concentration. They found no evidence that virus from nitrogen-deficient plants was reduced in infectivity over that from plants receiving ample nitrogen.

It is reported herein that in phosphorus experiments virus concentration coincided with the amount of phosphorus supplied to the host and not with the differential growth response of the host. With cucumber virus 1 in spinach (2) the growth response to increased phosphorus was quite variable. In some cases maximum growth was recorded at 93 p.p.m. while in others it occurred at 547 p.p.m. Regardless of which level showed maximum growth, virus concentration did not fall off with increased phosphorus. Results of these phosphorus studies are in close agreement with those of Bawden and Kassanis (1). In their sand cultures it was found that the highest concentration of tobacco mosaic virus in expressed sap was found at high phosphorus levels where growth was inhibited. In soil experiments, the highest level of phosphorus used was not sufficient to induce stunting.

In the present studies where nitrogen and phosphorus were simultaneously increased, virus concentration coincided with plant growth. At the high nutrient level both virus concentration and host growth were reduced. This suggests the possibility that, in the case of nitrogen, some metabolite at the high N levels may be serving as an inhibitor. This could not be detected by the local lesion assay and would explain why the same virus concentration curve was obtained from the nitrogen series and the nitrogen-phosphorus series but a different curve from that of phosphorus alone. This possibility is being further studied. It might also indicate that virus activity is more responsive to nitrogen variation than to phosphorus variation. This is at variance with Bawden and Kassanis (1).

The effects of potassium and concentration of balanced solutions on virus synthesis tend to emphasize the relation of host growth to virus activity. Potassium does not enter into the virus molecule so its effect on virus concentration would be expected to reflect response in host growth. Variations in supplies of potassium were found to have no marked effect on host growth and correspondingly no pronounced effect on virus activity. In general the results herein presented agree with the results of Cheo

et al. (2) in that any increase in host growth was paralleled by a corresponding increase in virus concentration. Bawden and Kassanis (1) suggested that increasing potassium caused a decrease in tobacco mosaic virus irrespective of its effect on host growth. Studies on the effect of concentration of balanced solutions on virus concentration showed that there was a positive correlation of host growth and virus activity.

SUMMARY

The influence of phosphorus, nitrogen, potassium and concentration of balanced solutions on plant growth and multiplication of turnip virus 1 in *Nicotiana glutinosa* L. and *N. multivalvis* Lindl. was studied by supplying them with nutrient solutions containing various amounts of the particular nutrient under study and measuring the virus activity of expressed sap by local lesion counts on half leaf inoculations to *Nicotiana tabacum* var. Connecticut Havana no. 38.

In nitrogen experiments the virus activity of expressed juice was directly correlated with plant growth. Plant growth increased with increased nitrogen up to 630 p.p.m. beyond which it declined. Plants growing in low-nitrogen solutions were chlorotic whereas plants growing in high-nitrogen solutions were very dark green. Virus assays showed that nitrogen levels that increased plant growth also increased virus concentration and vice versa. Experiments involving *N. multivalvis* revealed that the virus multiplication rate at high nitrogen levels was retarded but over the length of the experiments ultimate virus concentration exceeded that at the lower levels.

In phosphorus experiments growth of both *N. glutinosa* and *N. multivalvis* plants increased up to 237 p.p.m. beyond which it decreased. Virus activity of expressed sap closely paralleled the amount of phosphorus supplied to the host. Phosphorus levels sufficient to cause stunting did not inhibit virus multiplication but on the contrary, increased it. This effect on virus multiplication was apparent from the time of introduction of virus into the host throughout the disease cycle.

The effect of increasing nitrogen and phosphorus concurrently on host growth and virus multiplication was similar to the effect of increasing nitrogen alone. At the level where phosphorus and nitrogen were both in excess, growth and virus concentration were both reduced as was the case in nitrogen experiments. The excess phosphorus failed to overcome the inhibitory effect of the excess nitrogen.

Potassium variations had no marked effect on plant growth except where potassium was deficient or in excess and subsequently had little effect on virus concentration. Virus concentration was directly related to growth. Any increase in plant growth was accompanied by an increase in virus concentration and vice versa.

With variations in concentration of balanced solutions it was found that in general there was a positive correlation of host growth and virus concentration. Maximum growth was recorded at the basic level of 1H (N, P, K concentrations of 210, 31, and 235 p.p.m., respectively.) with marked stunting occurring at the higher concentrations. In *N. glutinosa* the virus multiplication rate was retarded at the higher concentrations but over a given period of time virus concentration at those levels equalled virus concentration at the lower levels where the best growth was recorded. Virus concentration in *N. multivalvis* coincided closely with plant growth.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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HEAT OF RESPIRATION OF INJURED AND DISEASED LEAVES[1]

C. E. Yarwood

All living tissues respire and evolve heat. The rate of heat evolution is due to and proportional to the rate of respiration, though not all energy of respiration is converted to heat. Injury or infection with pathogens increases the respiration and evolution of heat by plant tissues. Although these hypotheses may not be conclusively proven, much evidence in their support has been published. Further evidence is presented herein.

REVIEW OF LITERATURE.—Heat production by plants is not so well recognized as heat production by animals. An early record is that of Lamarck (Renaudet, 19) who in 1777 noted the high temperature of arum spathes. Subsequent records are those of Dutrochet (6), Richards (20), Pierce (18), Langsworthy and Milner (13), Molisch (16, 17), and Burton (2). No review of the literature on heat production by plants will be made here, and other references to the phenomenon are given in some of those cited.

The higher temperature of wounded than normal plant tissues was perhaps first reported by Richards (20), who also noted that potato tuber decay caused by unspecified organisms caused an increase in temperature. Evans and Evans (8) noticed that infection of living grapefruit by *Penicillium* caused an unspecified increase in temperature, but that development of *Penicillium* in dead host tissue caused no increase in temperature. The first case known to the writer of specified temperature increases by a specified pathogen is the report by Eglets (7) that potato tubers infected with *Bacillus phytophthorus* were as much as 0.16°C. above the temperature of normal tubers. Subsequent reports of temperature increase are: 0.12°C. for *Monilinia fructigena* in apple; 0.08°C. for *Botrytis cinerea* in apple; 0.07°C. for *Agrobacterium tumefaciens* in chrysanthemum, (9); 0.04°C. for *Phytophthora infestans* in potato; 0.05°C. for *Fusarium* in potato (10).

The greater heating of moist stored grain when heavily contaminated with microorganisms (11), and the secondary temperature rise associated with the growth of molds on leaves (16) might be considered as examples of increase in plant temperatures due to disease. A report by Berlese (1) in 1898 is cited by Chester (3) as an example of increased temperature due to a plant disease, but has not been seen by the writer.

The principal factors affecting leaf temperature are in Table 1. The most important are believed to be radiation and transpiration. Since the effect of disease on these factors is generally unknown, and since these factors may act simultaneously but in opposite directions, comparative temperature measurements of diseased and healthy leaves in a state of nature would be difficult to interpret. To measure the heat of respiration of leaves it is necessary to remove or greatly reduce such interfering factors as radiation and transpiration, which in a state of nature have a much greater effect on leaf temperature than does respiration.

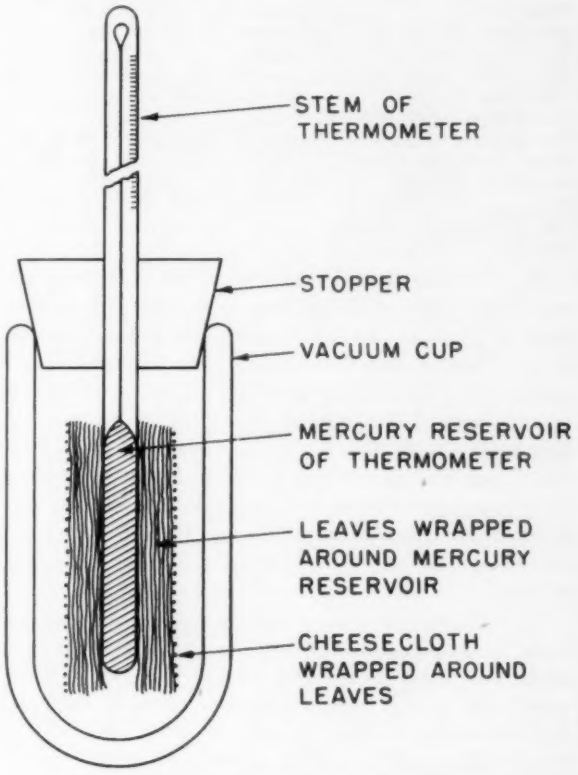


FIG. 1. Diagram of calorimeter used to measure evolution of heat by healthy and diseased leaves.

METHODS.—The temperature of leaves in stoppered vacuum cups (Fig. 1) was measured with mercury in glass thermometers reading to 0.01°C. The leaves from greenhouse or outdoor plants were wrapped snugly but without crushing around the mercury reservoirs of the thermometers and held in place by a wrapping of cheesecloth. Most of the trials were conducted in a laboratory where the temperature usually remained within 2° of 22°C. but some trials were at a constant temperature of 25°C. The temperatures of an empty vacuum cup, those of one or more containing healthy leaves, and those of one or more containing diseased leaves, were all observed simultaneously. The air temperature is that of the empty vacuum cup and the temperature increase is the difference between the empty vacuum cup and the one with leaves. The thermometers in water were found to have a maximum deviation of 0.03°C. from the lowest to the highest

[1] Accepted for publication August 27, 1953.

TABLE 1.—*Leaf temperatures*

Factor affecting leaf temperature	Effect on leaf temperature	Effect of infection on factor
radiation—to leaf	increases	unknown
radiation—from leaf	decreases	unknown
conduction—to leaf	increases	unknown
conduction—from leaf	decreases	unknown
convection—to leaf	increases	unknown
convection—from leaf	decreases	unknown
transpiration of leaf	decreases	increases or decreases
water absorption of leaf	increases	increases
respiration of leaf	increases	increases

reading.

To compare the performance of the different vacuum cups, 10cc. of warm water were added to cheesecloth wrappings around thermometer bulbs, and the temperature of these wrappings was followed for 5 successive periods of about 40 minutes each for 4 vacuum cups. The average deviation of the rate of heat escape from an individual cup for a single period was 7 per cent of the average rate of heat escape for all cups; the deviations were apparently randomly distributed for different vacuum cups for different periods. Therefore no correction was made for individual differences in thermometers or vacuum cups.

To determine the rate of heat loss from the vacuum cups, various amounts of warm water were added to them, and the temperatures of the vacuum cups of water and of an empty vacuum cup were followed simultaneously. Small amounts of water (5, 10, 15, and 25 cc.) were added to cheesecloth wrappings of the thermometer bulbs, but larger amounts of water were added to the bottoms of the cups. Temperatures were recorded at about 15 minute intervals. From data of this type the rate of heat loss was calculated as calories per hour per gram of water per degree centigrade of difference between the test and control vacuum cups (cal./hr./g/°C). For example, if 10 gm. of water at an original 2°C. above the control temperature, dropped to 1.5°C. above the control temperature in 30 minutes the apparent rate of heat loss would be $2(2\text{-}1.5)/(2+1.5)/2 = 0.67$ cal./hr./g/°C. From series of data of this type it is concluded that the rate of heat loss of a given amount of water (or leaves) expressed in the above units is approximately constant over the range of conditions of these trials after equilibrium is reached (about 1 hour). For all determinations of this type the rate of heat loss was about 0.9 cal./hr./g./°C. with 5 cc. of water, 0.61 cal. with 10 cc., 0.49 cal. with 15 cc., 0.41 cal. with 25 cc., 0.24 cal. with 50 cc., 0.18 cal. with 100 cc. and 0.08 cal. with 150 cc.

In this study fresh leaves will be considered to have a specific heat of 0.93. This is based on an average water content of about 90 per cent for the greenhouse-grown leaves, and a specific heat of 1 for water and 0.3 (14) for the dry matter of the leaves. Diseased leaves normally had a lower water content than healthy leaves, but this varied with age and intensity of infection and will usually not be corrected for.

The standard size of leaf samples was 10 gm. fresh weight and for such a sample the rate of heat loss is estimated as $0.93 \times 0.61 \times 10 = 5.7$ cal./hr./°C. The principal source of error in this estimate is believed to be any difference in the heat escape properties between wet cheesecloth and leaves.

From the above estimate of heat escape and from the observed temperature of the leaves it is possible to calculate the expected temperature of the leaves if there were no heat escape, and the rate of heat generation by the leaves. For 10 gm. of leaves the rate of heat escape in cal./hr./g./°C. would be 0.57× the observed leaf temperature increases. For larger or smaller amounts of leaves the appropriate constant would be used instead of 0.57. The calculated leaf temperature increase in °C. in the absence of heat escape would be the observed leaf temperature increase plus the cumulative loss of heat. The rate of heat generation in cal./hr./g. would be

$$0.93 \times \text{calculated leaf temperature increase}$$

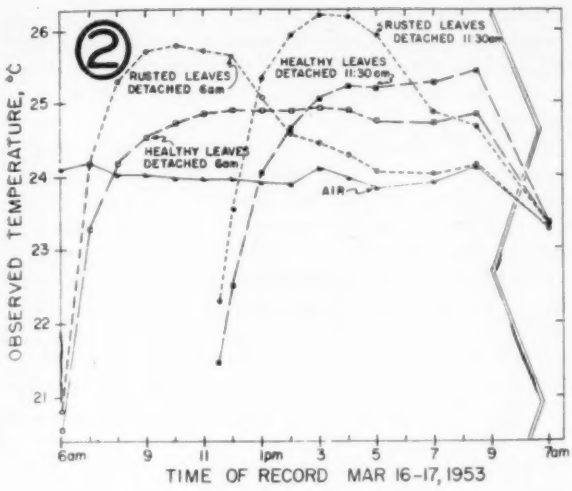
hours after 0°C. leaf temperature increase was reached.

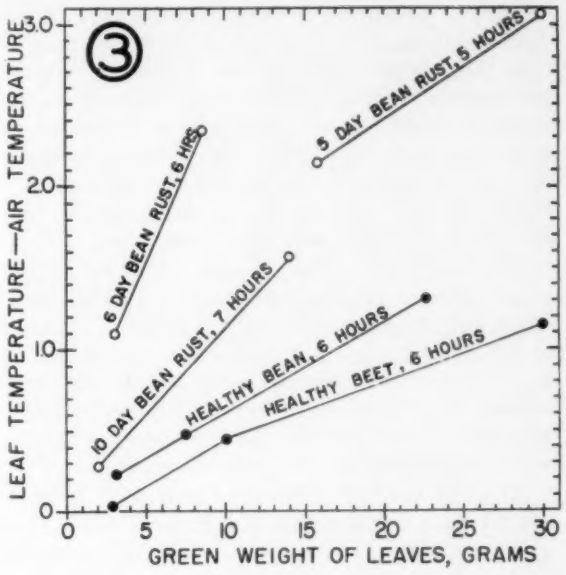
Diseased specimens were in most cases the product of greenhouse inoculations. Healthy specimens and those to be infected were chosen as comparable before inoculation. While the age of infection (time from inoculation to use) was generally known, the number of infections per unit area of leaf was usually not determined but was always high and in all cases perhaps 50 per cent or more of the leaf area was invaded by the pathogen.

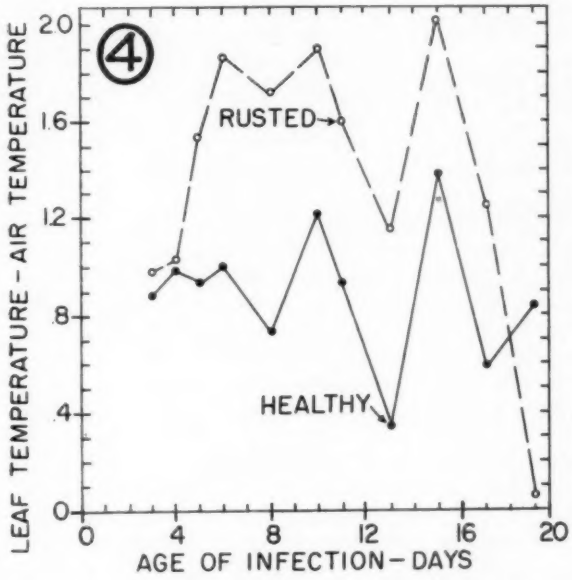
Respiration was measured by placing about 1 gm. green weight of leaves with 10 cc. 0.1 N KOH in a sealed pint jar for about 8 hours and then titrating the KOH in the control jars and in the jars with leaves. The leaves were oven dried to determine dry weight and the titration values were converted by conventional methods to mg. CO_2 evolved.

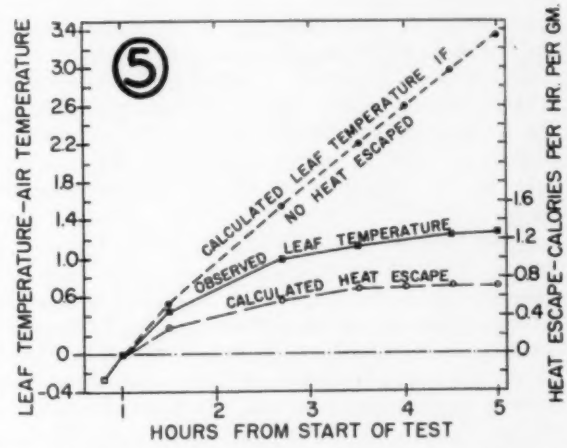
EXPERIMENTAL RESULTS.—*The course of leaf temperature in vacuum cups.*—Examples of the course of leaf temperatures in vacuum cups are given in Fig. 1. Initially, the temperature of leaves was always below the air temperature of the laboratory. This is presumed to be due to the cooling effect of transpiration in the absence of any intense source of radiation. The measured temperature of leaves in the vacuum cups rose rapidly to a maximum in 2–10 hours, and then fell more gradually. The rate of temperature rise before the maximum and the rate of fall after the maximum was consistently more rapid for diseased than for healthy leaves. The rapid rise is presumed to be due to the more intense initial respiration of the diseased tissue, and the more rapid fall is presumed to be due to the exhaustion of respiratory substrate and the accumulation of carbon dioxide.

For specimens kept more than 48 hours in the vacuum cups a second temperature rise and maximum was observed. This is presumed to be due to the influence of contaminating microorganisms, as observed




3
6 DAY BEAN RUST, 6 HRS.
5 DAY BEAN RUST, 5 HOURS
10 DAY BEAN RUST, 7 HOURS
HEALTHY BEAN, 6 HOURS
HEALTHY BEET, 6 HOURS
LEAF TEMPERATURE—AIR TEMPERATURE
GREEN WEIGHT OF LEAVES, GRAMS




5
CALCULATED LEAF TEMPERATURE IF NO HEAT ESCAPED
OBSERVED LEAF TEMPERATURE
CALCULATED HEAT ESCAPE
LEAF TEMPERATURE-AIR TEMPERATURE
HEAT ESCAPE-CALORIES PER HR. PER GM.
HOURS FROM START OF TEST

FIGS. 2–5. Fig. 2. The course of leaf temperatures in vacuum cups.—Fig. 3. Effect of size of leaf sample on leaf temperature in vacuum cups. The temperature increase was roughly proportional to the amount of leaves.—Fig. 4. Relation of age of rust infection to bean leaf temperature. In spite of the wide fluctuation in actual values, it is clear that infection had little effect on leaf temperature at 3–4 days after inoculation, and greatly increased leaf temperature at 5–17 days after inoculation. As the rusted leaves died (19 days after inoculation) their temperature became lower than that of healthy leaves.—Fig. 5. Observed leaf temperature, calculated heat escape, and calculated leaf temperature if no heat escaped for 10 gm. of rusted bean leaves in 1 trial. Methods of calculation are given in text.

by Molisch, (16). In the present study the maximum temperature increase reached in the first 10 hours is given as the leaf temperature increase. The maximum for the diseased leaves was usually reached before that of healthy leaves. When actual or relative differences in temperature between healthy and diseased leaves are given, the difference is between the respective maxima of each, even though these occur at different times.

Not infrequently, the apparent temperature of leaves held in the vacuum cups for 24 hours or more fell as much as 0.1°C. below that of the air for considerable periods. This is believed to be due to the insulating properties of the leaves under conditions of slowly rising temperature at a very low respiratory rate.

Effect of further insulation.—The temperature course of warm water and of bean leaves was followed in the standard vacuum cups exposed to the laboratory environment, and in these same vacuum cups surrounded by a further insulation of 3 in. of fine dry sawdust on all sides. With 150 cc. of water initially 4.3°C. above the air temperature the rate of heat escape was 0.062

cal./hr./g./°C. during 14 hours for the cups in sawdust, and 0.09 cal./hr./g./°C. for the normally exposed cups. With 8 gm. of rusted bean leaves the maximum temperature increase reached by the leaves in cups in sawdust was 1.63°C. and for the leaves in the normally exposed cups was 1.29°C. While an advantage of the extra insulation is apparent, it was not considered sufficiently great to justify the inconvenience, since the rate of heat loss could be corrected for.

Effect of size of leaf sample.—The temperature course of bean and beet leaf samples varying from 1 to 35 gm. in green weight was followed; results are in Fig. 2. Maximum leaf temperature was proportional to sample size. Increasing the sample size would increase slightly the accuracy of temperature determinations by this method, but 10 gm. green weight was arbitrarily chosen as the standard sample size.

Effect of position of leaves.—In 1 trial, 8 gm. of healthy bean leaves were arranged as an inside lining of a vacuum cup, and the cup closed with the stopper holding the thermometer so that the bulb did not touch the leaves. The twins of these leaves were used as a tight wrapping for another thermometer in the standard manner. Similarly 8 gm. of rusted bean leaves were arranged as a lining for a vacuum cup while their twins were wrapped around a thermometer bulb. The maximum observed temperature increases were 0.51°C. for the healthy leaves lining the cups, 0.62 for the healthy leaves wrapped around the thermometer bulbs, 0.82° for the rusted leaves lining the cups and 1.11 for the rusted leaves around the thermometer bulbs.

Effect of time of day.—The temperature course of 10 gm. sets of bean leaves harvested about dawn was compared with the temperature course of twin leaves harvested 4–6 hours later after exposure to considerable sunlight. Results of 1 such trial are in Fig. 1. In 4 such trials, healthy leaves harvested after exposure to several hours of light reached a 75 per cent higher temperature increase on the average than their twins harvested about dawn, and in the 2 trials rusted leaves harvested after several hours of light reached a temperature increase averaging 19 per cent higher than their twins harvested about dawn. Determinations in this study were usually started about 8 a.m.

Effect of added sugar.—In 1 trial bean plants were detached about dawn and placed with their stems in water, 0.1 per cent sucrose, 0.3 per cent sucrose, and 1 per cent sucrose in the dark for 3 hours, and then the leaves were detached and their temperatures were observed. Maximum temperature increases of 0.80, 0.89, 1.0, and 1.67°C. respectively were reached in about 3 hours, and of 0.39, 0.60, 0.70, and 0.60°C. in 15 hours. It is obvious that a high carbohydrate level caused higher leaf temperatures, presumably due to the greater respiration of the sugar-enriched leaves.

Effect of mechanical injury.—Two sets of 10 gm. of bean leaves each were rubbed with carborundum on their upper surfaces as in virus inoculations and their opposite twin leaves were maintained as controls. The maximum temperature increases for control and rubbed leaves were 0.63 and 0.72°C. for 1 paired comparison and 0.51 and 0.60 for the other paired comparison.

Effect of chemical injury.—Healthy bean leaves were sprayed with fungicides, and their temperatures taken immediately or after the spray material dried. In 1 trial when the leaves were placed in the vacuum cups immediately after spraying, the observed maximum leaf temperature increases were 0.55° for control dry leaves, 0.55° for leaves sprayed with water, 0.70° for leaves sprayed with 1 per cent bordeaux mixture, and 0.75 for leaves sprayed with 1 per cent lime sulfur. In 2 trials in which the leaves were sprayed with lime sulfur and dried before being placed in the vacuum cups, the average maximum temperature increases were 0.49° for water controls, 0.70 for 0.1 per cent lime sulfur, 0.80 for 0.3 per cent lime sulfur, 0.93 for 1 per cent lime sulfur, 0.95 for 3 per cent lime sulfur, and 0.97 for 10 per cent lime sulfur. For 1 series held in the vacuum cups for 24 hours the temperature increases were 0.55° for the control, 0.49° for 0.3 per cent lime sulfur, 0.43 for 1 per cent lime sulfur, and 0.38° for 3 per cent lime sulfur. This apparent reversal of temperatures with time is presumed to be due to the exhausting effect of the increased respiration during the early hours.

Effect of disease.—The effects of several diseases on leaf temperatures by the methods used are summarized in Table 2. With 14 out of 15 diseases, diseased leaves reached a higher temperature than healthy leaves. The average percentage increases in temperature due to disease were as follows: tobacco mosaic virus in tobacco—13, tobacco mosaic virus in *N. glutinosa*—18, tobacco necrosis virus in bean—35, southern bean mosaic virus in bean—47, white clover mosaic virus in bean—8.3, cabbage black ring virus in *N. glutinosa*—15, potato late blight—139, lettuce downy mildew—152, onion downy mildew—178, bean powdery mildew—37, oak powdery mildew—3, apple powdery mildew—23, bean anthracnose—146, and bean rust—71.

Data on leaf temperatures at different times after inoculation are in Table 2, but the effect of age of infection was investigated principally with bean rust (Fig. 3). The extreme fluctuation in the values for the controls on different days is disturbing, but may have been due in part to different weather and different ages of leaves in different trials. At 3 and 4 days after inoculation the observed increases in temperature due to rust infection are likely insignificant. From 5 to 17 days after inoculation the temperature increase due to disease ranged from 44 to 228 per cent above that of healthy leaves, but without a clear trend with time. This variation between trials may have been partly due to differences in the number of rust pustules per unit area of leaves. At 19 days after inoculation the rusted leaves were almost dead and their low temperature was undoubtedly due to their low rate of respiration.

TABLE 2.—*Temperature of 10 grams of insulated leaves*

Pathogen, host, and type of infection	Age of infection days [a]	Leaf temperature minus air temperature °C.	
		Healthy	Diseased
Tobacco mosaic virus on *Nicotiana tabacum*, systemic	15(1) [b]	0.60	0.73
Tobacco mosaic virus on *Nicotiana tabacum*, systemic	20(5)	0.95	1.00
Tobacco mosaic virus on *Nicotiana tabacum*, systemic	25(3)	0.91	1.13
Tobacco mosaic virus on *Nicotiana glutinosa*, local	3(1)	1.60	1.92
Tobacco mosaic virus on *Nicotiana glutinosa*, local	6(1)	0.83	1.05
Tobacco mosaic virus on *Nicotiana glutinosa*, local	13(1)	0.57	1.23
Tobacco mosaic virus on *Nicotiana glutinosa*, local	13(1) [c]	0.69	0.16
Tobacco necrosis virus on *Phaseolus vulgaris*, local	1.5(1)	0.56	0.84
Tobacco necrosis virus on *Phaseolus vulgaris*, local	2.0(1)	1.15	2.01
Tobacco necrosis virus on *Phaseolus vulgaris*, local	3(1)	0.98	1.08
Tobacco necrosis virus on *Phaseolus vulgaris*, local	4(1)	1.13	1.32
Tobacco necrosis virus on *Phaseolus vulgaris*, local	5(1)	0.99	1.23
Southern bean mosaic virus on *Phaseolus vulgaris*, local	21(1) [d]	0.64	1.24
Southern bean mosaic virus on *Phaseolus vulgaris*, systemic	30(1) [e]	1.05	1.24
White Clover mosaic virus on *Phaseolus vulgaris*, local	9(2)	0.81	0.88
White Clover mosaic virus on *Phaseolus vulgaris*, local	15(1)	0.75	0.81
Cabbage black ring virus on *Nicotiana glutinosa*, systemic	17(1)	1.74	1.99
Phytophthora infestans on *Solanum tuberosum*, local	6(1)	1.01	2.41
Bremia lactucae on *Lactuca sativa*, local	6(2)	0.63	1.59
Peronospora destructor on *Allium cepa*, local	10(1)	0.84	2.42
Erysiphe polygoni on *Phaseolus vulgaris*, local	10(1)	0.90	1.23
Sphaerotheca lanestris, on *Quercus agrifolia*, systemic	(1) [f]	2.50	2.57
Podosphaera leucotricha on *Pyrus malus*, systemic	(1) [f]	2.10	2.59
Taphrina deformans on *Prunus persica*	(1) [f]	2.07	1.79
Colletotrichum lindemuthianum on *Phaseolus vulgaris*, local	5(1)	0.85	2.09
Uromyces phaseoli on *Phaseolus vulgaris*, local	3 to 19(23) [g]	0.89	1.52

[a] Time from inoculation to detachment and temperature measurement.
[b] Number in parenthesis is number of trials.
[c] Heavier infection than above, infected leaves mostly dead.
[d] Inoculated primary leaves with chlorotic lesions.
[e] Systemically infected secondary leaves.
[f] Natural infection.
[g] More detailed data in Fig. 3 and text.

Effect of the living pathogen.—The temperatures of both healthy and rusted bean leaves which had been heated at 45°C. for 90 seconds to kill the pathogen without apparent host injury (21), were compared simultaneously with unheated leaves in 2 tests. The average leaf temperature increases were as follows: healthy—1.19°C., heated healthy—1.01, rusted—1.86, heated rusted—1.69. It appears that the killing of the rust organism did not cause a greater reduction of leaf temperature than a similar treatment of healthy leaves. The increase in temperature of rusted over healthy leaves would appear to be primarily due to the infection-induced host respiration, rather than to the respiration of the rust fungus. The apparent decrease in temperature of healthy and rusted leaves after exposure to heat may have been due to the early stimulation of respiration with resulting exhaustion of carbohydrates.

Relation to respiration.—The relation of respiration to leaf temperature was not followed by simultaneous observations of respiration and temperature on the same material, but independent data on respiration of similar material seem sufficiently closely related to present here. Average values as mg. of carbon dioxide per gm. dry weight of leaves per hour (number of replications in parenthesis) were as follows: bean—2.16(12), cucumber—2.03(2), cantaloupe—2.56(3), and onion 1.29(11). The increases in respiration due to treatment were as follows: 49 per cent for 1 per cent lime sulfur spray on bean, 21 per cent for powdery mildew on bean, 10 per cent for downy mildew on cucumber, 76 per cent for 1 per cent lime sulfur on cantaloupe, and 56 per cent for downy mildew on onion. The correlation of these values with those of temperature (Table 2) does not establish a close numerical relation between increase in respiration and in temperature, but combined with the data of Richards (20), Eglets (7), DuBois (5), and Fischer (9), and others, supports the hypothesis that temperature increase may be roughly proportional to the increase in respiration.

Total heat evolved.—Most of the heat evolved by the time the maximum observed leaf temperature had been reached had escaped from the container. From calculations of the rate of heat escape as given under the discussion of methods, the expected temperature, if there had been no heat escape, has been calculated for 1 trial of rusted bean leaves 8 days after inoculation (Fig. 4). Although the observed leaf temperature had

stopped rising at about 5 hours in this test, the calculated temperature, if there had been no heat escape, continued to rise and would have been about 3.4°C. when the observed temperature was 1.28°C. The observed temperature under the conditions of these tests has only relative significance and is determined by the insulating properties of the container.

The total heat evolved can be calculated from the observed leaf temperature plus the escaped heat or from the leaf temperatures expected if no heat had escaped. In 4 hours (from 1 to 5 hours after start of test) the leaves of Fig. 3 produced $9.3 \times 3.39 = 31.5$ calories or 3.15 calories per gram or 0.76 calories per gm. per hour. Leaves of this type can be expected to respire at the rate of about 0.4 mg. CO_2 per gram green weight. Since 0.4 mg. CO_2 in aerobic respiration with a respiratory quotient of 1 represents about 1 calorie of heat if all respiration energy were converted to heat (4), it would appear from these rough calculations that most of the energy of respiration under these conditions had been converted to heat. A similar conclusion was reached by Langsworthy and Milner (13).

Discussion.—Because of differences in method and material it is difficult to compare the results of this study with the results of previous studies. The greater heats of respiration observed here with leaves than observed by Eglets (7) with potato tubers and Fischer (9) with apple fruits and potato tubers are undoubtedly due largely to the greater respiration rates of leaves than of storage organs. The average rate of respiration of healthy leaves in this study was about 2 mg. CO_2/g./hr. at about 25°C., while the respiration rate of potato tubers is about 0.05 mg. CO_2/g./hr. (15) and of apple fruits is about 0.3 mg. CO_2/g./hr. (12). The temperatures of healthy tissues studied by Eglets and Fischer were so low and variable as to be probably insignificant, but if we accept 0.005°C. as the temperature of healthy potato tissue (10), then the maximum increase in temperature due to infection with *Bacillus phytophthorus* (7) is about 33-fold, or much greater than observed in this study. The increases in respiration due to disease were also much greater as observed by Eglets and Fischer than as observed in this study. It would appear that absolute increases in respiration and temperature due to disease are much greater in leaves than in potato tubers or apple fruits, but the relative increase is much greater in potatoes and apples than in leaves.

Peach leaf curl was the only case studied where diseased leaves apparently had a lower temperature than healthy leaves. This may well be because the dry matter of curled leaves averaged only about 20 per cent of their green weight while the dry matter content of healthy leaves was about 34 per cent. With most foliage diseases studied by the writer, and with all others of the present study, the dry matter content of diseased leaves was greater than that of healthy (data not presented). The low dry matter content of curled peach leaves could mean a lower respiratory substrate, and consequently lower respiration per unit green weight. If leaf samples had been chosen to be of the same dry matter content, the available evidence indicates that the temperature of curled leaves would be greater than that of healthy. This is not to suggest that the increased respiration of diseased tissues in general is due to their higher dry matter content, for in all cases studied the increase in temperature was greater than the increase in dry weight.

SUMMARY

The temperatures of 10 gm. green weight of detached healthy leaves in vacuum cups reached 0.5–2.5°C. above air temperature in 4–20 hours. The observed leaf temperatures increased with further insulation, and with increased size of leaf sample, was greater for leaves taken late than early in the day, and was greater for leaves given an extra supply of sugar. Leaves which had been rubbed with carborundum or sprayed with lime sulfur or Bordeaux mixture reached a higher temperature than untreated leaves. Separate infection of leaves with 5 different viruses (tobacco mosaic, tobacco necrosis, southern bean mosaic, white clover mosaic, and cabbage black ring) increased leaf temperatures by an average of 21 per cent. Separate infection of leaves with 9 different fungi (*Phytophthora infestans, Bremia lactucae, Peronospora destructor, Erysiphe polygoni, Sphaerotheca lanestris, Podosphaera leucotricha, Taphrina deformans, Colletotrichum lindemuthianum* and *Uromyces phaseoli*) increased leaf temperatures by an average of 82 per cent. Only leaf curl-infected peach leaves showed a lower temperature than healthy leaves, probably an anomaly due to the low dry matter content of infected peach leaves. With diseased leaves the rise in temperature after leaves were placed in the vacuum cups and the fall in temperature after the maximum was reached was greater than for healthy leaves. Temperatures of rusted bean leaves rose clearly above that of healthy leaves at 5 days after infection, remained 44–228 per cent above healthy leaves until 17 days after infection, and fell below that of healthy leaves with death of the leaves at about 19 days after infection. Since the killing of bean rust mycelium in living leaves did not cause a clear decrease in leaf temperature, it is tentatively concluded that the increased temperature is caused principally by host respiration rather than by the respiration of the pathogen. Rough calculations indicate that most of the energy of respiration of leaves under conditions of these tests is liberated as heat.

Department of Plant Pathology
University of California
Berkeley, California

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FURTHER STUDIES ON PHYSIOLOGIC RACES OF USTILAGO TRITICI [1]

Wayne M. Bever [2]

The writer described 11 races of loose smut of wheat (*Ustilago tritici* (Pers.) Rostr.)[3] in 1947. Most of these races were derived from inoculum collected before 1939 in the area described by Clark and Bayles[4] as the eastern wheat growing region. The writer and B. B. Bayles made additional smut collections from most of that region in 1947 for studying the epidemiology and any possible new races of the loose smut fungus.

MATERIALS AND METHODS.—The technic of inoculation and the differential hosts in this study were the same as previously employed. Kangip (C.I. 13094),[5] a selection from Kanred-Gipsy (C.I. 11382), has been used continuously since the beginning of the loose-smut physiologic race studies, but formerly was listed under the designation of the unselected strain.

The original smut inoculum was stored in a refrigerator at 0°–4° C. until it was used to inoculate the differential varieties. For subsequent tests it was collected from the same differential variety each year and stored in the refrigerator until used.

A smut collection that could not be definitely established as a race by the reaction of the differential varieties in the first year's test was tested further until a definite host-reaction pattern was established. In all, 56 collections from Texas, Missouri, Nebraska, and states to the eastward were tested. These were identified as already existing races or as new races based on the reaction of the differential varieties grown from infected seed produced from plants inoculated in the greenhouse. The 11 previously described races were included in the study for comparison.

Seed from 4 heads of each of the differential varieties that had been inoculated with the same smut collection was thoroughly mixed and divided into 4 lots of 12–30 seeds each, depending on the amount available. In the fall these lots were sown in separate hills as described by Bonnett and Bever.[6] The hills were spaced 6 in. apart in rows 20 in. apart. The percentages of smut were based on the total number of heads in 4 hills, which ranged from 100 to 420.

The races are designated numerically. Infection percentages are given in the nearest whole number. As in previous separations of loose smut races, any variety with 10 per cent smut infection or less was

[1] Accepted for publication August 21, 1953.
Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Illinois Agricultural Experiment Station.

[2] Agent (Pathologist), Division of Cereal Crops and Diseases and Professor of Crop Pathology, Department of Agronomy, University of Illinois.

[3] Bever, W. M. 1947. Physiologic races of Ustilago tritici in the eastern soft wheat region of the United States. Phytopathology 27: 889-895.

[4] Clark, J. A., and B. B. Bayles. 1951. Distribution of varieties and classes of wheat in the United States in 1949. U. S. Dept. Agr. Cir. No. 861.

[5] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

[6] Bonnett, O. T., and W. M. Bever. 1947. Head-hill method of planting head selections of small grains. Jour. Amer. Soc. Agron. 39: 442-445.

TABLE 1.—*Average percentage of smutted heads in 10 varieties of winter wheat inoculated with 11 previously described races and 8 new races of Ustilago tritici*

	Average percentage of smutted heads in									
	Wabash (C.I. 11384)	Am. Banner (C.I. 6943)	Purdue #1 (C.I. 11380)	Hussar (C.I. 4843)	E. Premium (C.I. 11858)	Nabob (C.I. 8869)	Forward (C.I. 6691)	Trumbull (C.I. 5657)	Kangip (C.I. 13094)	Leap (C.I. 4823)
Physiologic race no.										
1	73	0	0	0	0	0	0	0	0	0
2	27	34	43	18	26	0	0	0	0	0
3	45	23	29	20	41	15	0	0	0	0
4	54	51	34	16	42	34	50	0	0	0
5	34	20	43	12	26	20	32	18	0	0
6	47	72	38	30	53	21	42	51	60	53
7	58	55	45	35	39	43	28	58	0	29
8	55	8	34	13	9	4	21	30	0	0
9	58	0	20	0	8	0	0	36	0	0
10	68	0	5	3	0	0	0	4	25	0
11	1	21	0	24	0	0	0	0	0	0
12	5	32	2	1	1	0	2	1	2	29
13	35	0	29	0	61	2	30	30	36	70
14	45	7	21	21	47	2	31	30	20	41
15	53	12	2	0	0	0	51	0	1	27
16	47	33	33	0	42	4	28	26	6	34
17	36	33	5	2	43	2	29	28	8	32
18	29	36	31	11	30	26	21	0	0	43
19	42	0	0	0	0	0	42	0	0	0

considered resistant and those with 11 per cent or more were considered susceptible.

RESULTS.—Of the 56 smut collections tested, 5 proved to be race 1, 2 race 6, and 1 race 7. The re-

TABLE 2.—*Source of 11 previously described and 8 new physiologic races of Ustilago tritici*

Physiologic race no.	Collection numbers	Source of original collections (variety and state)
1	9, 10, 30, 40, 50, 51, 54, 59, 65, 84, 87, 47-31, 47-38, 47-28, 48-16	Wabash, Purplestraw, Fultz, Fulcaster, Posey's Bluestem, Red Rock, Goens, Durum from Ga., Ill., Ind., Ky., Mich., Mo., Nebr., Tex.
2	13, 25, 33, 47	Purplestraw, Honor, Forward, Clarkan from Ga., N.C., N.Y.
3	1, 4, 14, 15, 17, 18, 19, 21, 31, 38	Purplestraw, Fulcaster, Mediterranean, Grandprize from Ga., Ky., N.C., S.C., Tex.
4	41	Goens from Ky.
5	2, 37	Purplestraw, Fultz from Ga., S.C., Tex.
6	11, 12, 23, 61, 97, 107	Brill, Purplestraw, Illinois #2, Early Premium from Ga., Ill., Md., N.C.
7	28, 32, 34, 37, 44, 102, Mo. 3	Fulcaster, Purplestraw, Trumbull from Ill., Ky., Mo., N.C., S.C., Tenn., Va.
8	24	Forward from N.C.
9	8	Fultz from Tex.
10	52, 53, 55, 66, 69, 72, 109, 112	Composite of varieties, Fulcaster, Fultz, Hope-Turkey, Red May from Ark., Ind., Tex.
11	29, 60, 74	Turkey and two mixed varieties from Ill., Ohio, Tex.
12	47-10, 47-36, 47-40, 47-42, 47-45, 47-48, 47-50, 47-51, 48-9, 48-10, 48-12	Malakof x P44, Yorkwin, Cornell 595, Composite (12 fields), from Mich., Mo., N.Y., Penn., S.C.
13	49-17, 49-18, 49-19, 49-20, 49-27, 49-28	Vahart (4 coll.), Leap, unnamed variety from Va.
14	99, 48-4a, 48-13, 48-17, 48-19, 48-20	Yorkwin, Unnamed variety, Fultz from Nebr., N.Y., Tex.
15	49-6, 49-8, 49-11	Fultz, Rudy, Redhart from Ill., Miss.
16	49-7	Vigo from Illinois
17	108, 49-3, 50-1	Berkeley Rock, Coker 47-23 from Ala., Ill., Tex.
18	103, 48-5	Unnamed variety, Yorkwin from N.C., N.Y.
19	48-38, 48-6, 48-7, 49-1, 49-9, 49-10	Mediterranean, Fultz, Norwin #10, Rudy, Fulcaster from Ill., Ind., Tex.

mainder constituted 8 entirely new races according to the reaction of the differential varieties. The average percentages of smutted heads produced by the different races in the differential varieties are in Table 1. Races 12 to 19 are new ones. Most of the new races are distinguished from the old races by the reaction of 2 or more differential varieties. Kawvale (C.I. 8180), not listed in Table 1, is resistant to all races.

The wheat varieties and states from which the races were originally collected are in Table 2. It will be noted that the new races 12 to 19 were collected almost entirely on wheat varieties different from those on which the 11 original races were collected. The new varieties with different germ plasm may partly explain the appearance of so many new races within the area in a relatively short period of time. Although no specific studies have been made on the frequency of mutation or natural crossing in *Ustilago tritici*, it is generally assumed that little of either occurs under natural conditions. This assumption is based on the rather unusual life cycle of the loose smut fungus.

With the exception of race 16, all the new races were collected more than once.

The distinguishing characteristics of physiologic races 12 to 19 are in Table 3. Races 1 to 11 were described previously.[3]

TABLE 3.—*Distinguishing characteristics of races 12 to 19 of Ustilago tritici*

Physiologic race no.	Distinguishing characteristics of races
12	Differs from race 11 in that Hussar is resistant and Leap is susceptible.
13	Differs from race 12 in that Wabash, Purdue #1, Early Premium, Forward, Trumbull and Kangip are susceptible. Differs from race 9 in that Early Premium, Forward and Kangip are susceptible.
14	Similar to race 13 but differs in that Hussar is susceptible.
15	Differs from race 14 in that Purdue #1, Hussar, Early Premium, Trumbull and Kangip are resistant.
16	Similar to race 15 but differs in that Purdue #1, Early Premium, and Trumbull are susceptible.
17	Similar to race 16 but differs in that Purdue #1 is resistant.
18	Differs from race 17 in that Purdue #1 and Nabob are susceptible and Trumbull is resistant.
19	Differs from races 11 and 12 in that Wabash and Trumbull are susceptible; all others resistant.

DISCUSSION.—Fifty-six collections of *Ustilago tritici* made in the soft winter wheat region in 1947 contained 3 previously described races 1, 6, and 7, and 8 new races, identified on the basis of pathogenicity tests on 10 varieties of winter wheat. Race 1 continues to be the most prevalent and widely distributed race described thus far. It has been isolated from 14 different smut collections.

The appearance of 8 new loose-smut races since 1939 demonstrates the influence of new wheat germ plasm on the shift in prevalence of physiologic races within a given area. Nearly all the collections in 1947 were from varieties not generally grown in 1939. Eight of the 11 older races were not even isolated from the inoculum collected in 1947, indicating that they were rare or no longer present.

Since Kawvale is resistant to all races thus far described, it should serve as an excellent parent in breeding for resistance to loose smut. Kawvale has smutted as much as 25 per cent in occasional experiments, but little or no smut has appeared when inoculum collected from Kawvale was used to reinoculate the variety. The reason for this reaction has not been established.

SUMMARY

Eight new races of *Ustilago tritici* were isolated from 56 loose-smut collections made in 1947. A total of 19 races have been described. Race 1 is still the most prevalent.

The results indicate that new varieties of wheat, constituting different germ plasm, shift the prevalence of the different physiologic races of *U. tritici*.

Kawvale is resistant to all races and should be an excellent parent in breeding for resistance to loose smut.

DEPARTMENT OF AGRONOMY, UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

IDENTITY OF POTATO CURLY TOP AND GREEN DWARF [1]

J. D. Menzies and N. J. Giddings

A virus disease of potatoes called green dwarf was described by Milbrath in Oregon in 1946 (9). He reported its occurrence in the irrigated areas of eastern Oregon where it was causing concern to potato seed producers. The disease subsequently was identified in the Yakima Valley of Washington when it was found to be a confusing factor in potato leaf roll investigations at the Irrigation Experiment Station, Prosser. This paper reports the joint studies of the authors leading to the conclusion that potato green dwarf is caused by a previously undescribed strain of the curly top virus. Preliminary results have already been published (8).

LITERATURE REVIEW.—Despite the fact that curly top virus occurs in the western states on many vegetables and ornamental crops including such solanaceous crops as tomato and pepper, until recently it has been found only rarely in potato. Severin (10) in 1929 reported the field occurrence of a disease in potatoes in California from which the curly top virus was recovered by previously non-viruliferous beet leafhoppers. In 1940, Jones, Vincent, and Burke (5) described curly top affecting seedling potato varieties grown in test plots at Pullman, Washington. Giddings (1) in 1947 confirmed Severin's results by inoculating young potato seedlings but noted that 91 separate tests for curly top from suspected plants in commercial fields were negative. He recently reported (2) considerable curly top damage in some southern California fields where potatoes were being grown for certified seed.

GRAFT TRANSMISSION EXPERIMENTS.—The relationship between green dwarf and curly top was first suspected when tomato was used as a test species in a study of yellows type viruses in potato. It was found that when potatoes with green dwarf symptoms were grafted to tomato a disease reaction very similar to curly top resulted (Fig. 1C). Milbrath visited the experimental plots and identified the potato disease in question as the green dwarf that he had described in 1946.

Symptoms induced in tomato test plants by grafting with green dwarf infected potato scions were carefully compared with those induced by scions from field grown tomato plants affected with curly top. The two diseases produced identical symptoms in all cases.

Because of the confusing similarity of symptoms induced in potato by curly top, leaf roll and certain other viruses, the reaction of tomato to these viruses is important in diagnosis. These 2 species form reciprocal graft unions very readily by the procedure used in the above tests. The details of the method are as follows:

Healthy uniform tomato plants of the variety Stokesdale are used when about 6 in. high. Terminal scions from the potato plant to be tested are inserted as wedge grafts in the tomato stem at about the second leaf node. The cut in the tomato stem is made from a point ¼ in. above the leaf axil to near the center of the stem 1 in. below the node. The scion is matched with the stock at 1 edge of the graft and the union bound with a standard fruit-tree budding rubber. The graft is tied so that the tomato leaf and its axillary bud protrude between the laps of the rubber binding. After grafting, the plants are placed in a glass moist chamber for 6 days, then removed to a greenhouse bench. At this time the tomato may be cut back to a point just above the graft. The new shoots thus forced from the leaf axils will show disease symptoms more rapidly than the terminal shoot. With this method successful grafts have been obtained consistently on tomato from potato, pepper, *Physalis angulata* L. and *Datura stramonium* L. If the cambium tissues of the wedge-cut scion are matched at 1 edge with those of the stock, the relative size of stock and scion is not important. Small spindly shoots from infected potato tubers and thin woody pepper scions, for example, can be readily grafted to tomato.

INSECT TRANSMISSION EXPERIMENTS.—In 1950 several tomato plants graft-infected with green dwarf were sent to the Division of Sugar Plant Investigations laboratory at Riverside, California for tests with sugar beet leafhoppers (*Circulifer tenellus*), the only insect vector known for curly top. Curly top virus was obtained from each of these plants and the virus from them was inoculated into large sugar beet plants to serve as stock cultures. One of these selections was designated as curly top virus strain 12 and was used in several groups of inoculations of potato plants grown from certified Russet Burbank tubers. Infection was readily induced, over 50 per cent of the plants becoming infected when 5 or more viruliferous leafhoppers per plant were used. Two of these plants produced small tubers which were replanted after 4 months cold storage. Plants from them grew very slowly and some developed symptoms of green dwarf while occasionally 1 developed many sprouts, giving a witches-broom type of reaction. Curly top virus was recovered from plants showing each type of symptom. Some of the seed pieces remained in an almost completely dormant condition for 2–4 months before sprouting.

Again in 1951 both tubers from green dwarf infected plants and tomato plants graft-infected with green dwarf were sent from Prosser to Riverside. The curly

[1] Accepted for publication August 10, 1953.
Cooperative investigations between Division of Soil Management—Irrigated and Dry Land Regions, U. S. Department of Agriculture, Irrigation Experiment Station, Prosser, Washington, and Division of Sugar Plant Investigations, U. S. Department of Agriculture, Riverside, California.

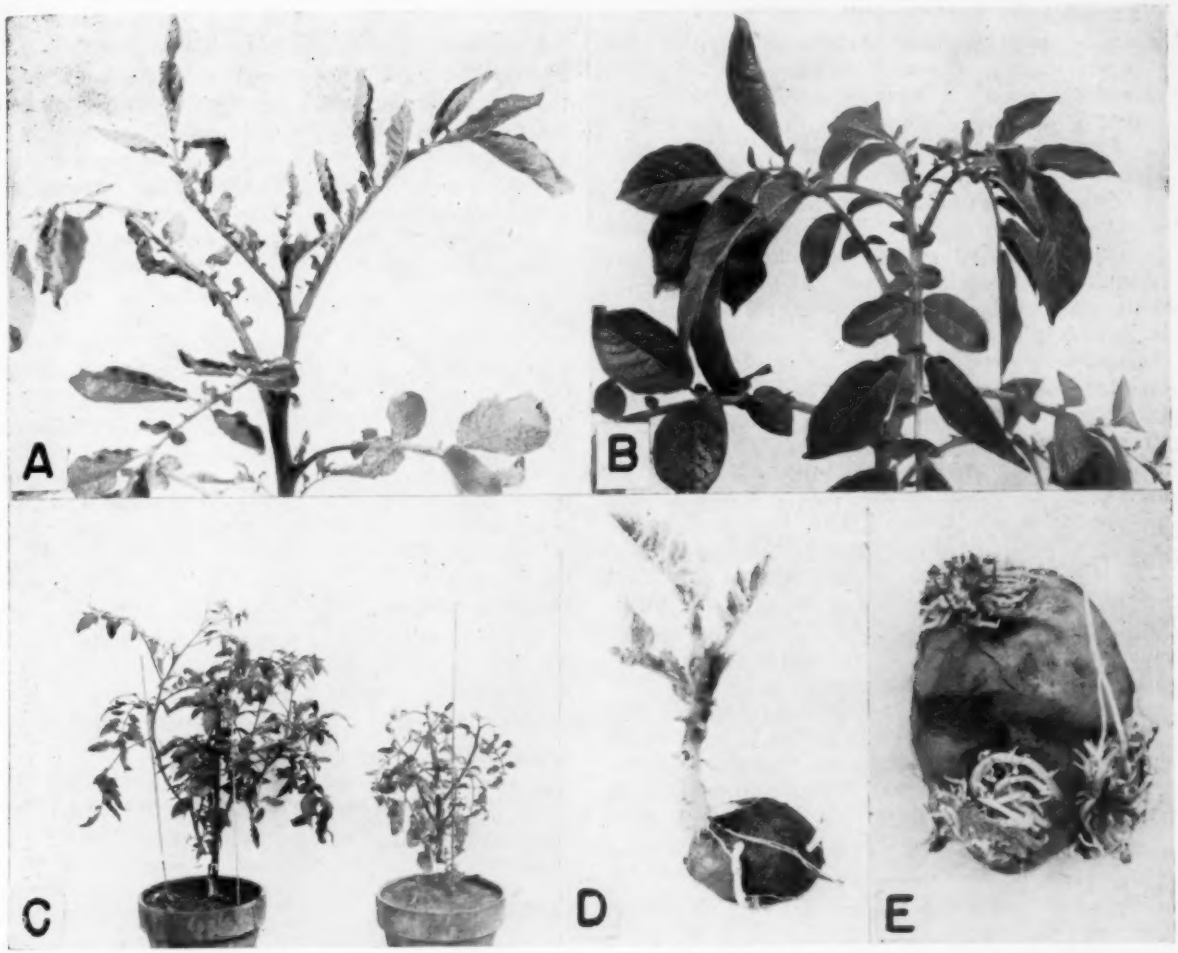

FIG. 1. A) Typical symptoms of current season curly top infection in Russet Burbank variety of potato; B) Healthy shoot of same variety and age; C) RIGHT: Curly top symptoms in Stokesdale tomato resulting from graft inoculation with potato to green dwarf. LEFT: uninoculated control; D) Tuber transmitted symptoms of curly top in Russet Burbank potato; E) Semi-dormant seed tuber, photographed at normal harvest time, showing development of numerous dwarfed shoots.

top virus was obtained from each.

The results of the graft and insect transmission experiments show that the potato disease known as green dwarf is actually curly top. The curly top virus strain which induces this disease is described in another publication (3).

FIELD OBSERVATIONS.—Current season symptoms of curly top in the commonly grown Russet Burbank variety are quite distinctive under Eastern Washington conditions. The most dependable of these symptoms is the ruffled, "pinched in" terminal growth shown in Fig. 1,A. In later stages the plant is stunted, with stiff, erect leaf petioles and lateral shoots. The color changes from normal to a pale olive green during the course of infection.

Current season's symptoms have been produced in the greenhouse following graft inoculation but only after the plants were pruned back to force new shoot growth.

Tuber-transmitted symptoms vary widely, particularly where seedling varieties are concerned and are readily confused with those of other diseases. The dwarfed shoots may remain green or display ranges of yellow, red and bronze pigmentation. Seed-piece dormancy may be complete or may be expressed as late-appearing, very dwarfed shoots that often fail to reach the soil surface (Fig. 1, D and E). These dormant or semi-dormant seed pieces are very firm and seem to resist decay. This symptom, however, is not specific for green dwarf. Milbrath (9) has illustrated typical dwarfed shoots and has described seed-piece dormancy.

Curly top of potato is not at present a disease of much economic importance in Washington. As high as 5 per cent infection may be found in commercial fields but this is unusual. Early planted potatoes are more likely to be infected than late planted potatoes. This may be due to the early season migration of the vector, the beet leafhopper. In this area curly top susceptible vegetables can often be grown successfully when planted in mid-summer, after most of the vector migration is over.

Exceptionally heavy infection has been obtained in experimental plantings grown near diseased tomatoes and sugar beets. In 1 experiment potato plants spaced 3 ft. apart near tomatoes developed 34 per cent curly top during the 1951 season. Yield records from 68 of these infected hills compared to a like number of randomly-selected healthy plants showed that the average current-season infected curly top plants yielded 52 per cent of normal. The range, however, was from no tubers whatever to a normal crop, depending upon the earliness of infection.

DISCUSSION.—So far as is known, potato curly top is limited in distribution to the northern portion of the curly top area of western United States and an area in southern California. The fact that it has been known in the northwest for so long suggests that this strain of curly top has originated in the northwest and has not yet become distributed over the entire curly top range. It is probably too soon to decide whether the present unimportance of the disease in this area is due to restricted distribution of the green dwarf strain or to the avoidance of potatoes as a food plant by the leafhopper vector. Infection of young plants by this strain using only 5 viruliferous leafhoppers indicates that a favorable combination of conditions might result in considerable damage to potatoes.

The bunch-top disease of potatoes reported by McLeod (6) in New Brunswick is similar to curly top in that it has a dormant seed-piece symptom and, on grafting, induces symptoms in tomato resembling curly top. However, it is unlikely that the curly top virus could be causing this disease so far east of its normal range. Seed-piece dormancy similar to that induced by curly top virus has also been found associated with the tomato big bud virus in potato (7). The potato-rosette disease in Tasmania is reported by Hutton and Oldaker (4) to be very similar to potato green dwarf as described by Milbrath. The reported symptoms, induced by grafting to tomato, however, are not typical of curly top and the curly top virus is not known to occur in Tasmania.

SUMMARY

The disease previously described as potato green dwarf is caused by a strain of the curly top virus. Graft transmission to tomato results in typical curly top symptoms in tomato. The virus has been transmitted from potato and tomato to sugar beets by the beet leafhopper. Reinoculation from sugar beet to potato produces typical green dwarf symptoms. In conformity with the established custom of assigning the common name "curly top" to diseases caused by the sugar beet curly top virus, the disease in potato should now be designated as potato curly top.

The curly top strain, designated as strain 12 is known to occur in Oregon, Washington and California. The disease in potatoes is sometimes of economic importance in fields being grown for certified seed-stock.

IRRIGATION EXPERIMENT STATION
PROSSER, WASHINGTON, AND
UNITED STATES SUGAR PLANT FIELD LABORATORY
RIVERSIDE, CALIFORNIA

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FURTHER STUDIES ON THE ELECTRON MICROSCOPY OF PURIFIED TOBACCO RINGSPOT VIRUS [1]

P. R. Desjardins,[2] C. A. Senseney, and G. E. Hess

Values varying from 13 to 26 mμ have been reported for the diameter of tobacco ringspot virus particles as determined by various methods (2, 6, 9, 10). The work described below was undertaken in order to establish the particle size and shape with more precision. During the course of this work characteristic particles were found in preparations from healthy tissue which were somewhat similar in shape and size range to those in preparations from diseased tissue. Efforts have, therefore, been made to obtain the virus in a higher state of purity, to correlate the presence of the characteristic particles with the presence of the infective agent, and to note any differences that appear between the virus and normal particles.

MATERIALS AND METHODS.—*Growth and preliminary purification of virus.*—Plant juice from virus-free and tobacco ringspot infected tobacco plants (Posey's Medium Broadleaf variety) was clarified by the following procedure. Leaves showing typical ringspot symptoms were harvested 14 days after inoculation and then were minced. Sufficient dipotassium phosphate was added to bring the final concentration to 3 per cent by weight of the minced tissue. The resulting mixture was allowed to stand at 4°C. for 1 hour, and the juice then pressed through 8 folds of cheesecloth. Final clarification was accomplished by centrifuging the expressed juice for 30 minutes at 1800 × G in an International centrifuge.

The initial concentration and purification of the virus was achieved by the differential centrifugation technique described in the literature (1, 7, 8) and used by Stanley (6) in the purification of tobacco ringspot virus. The virus pellets resulting from the high speed centrifugation were resuspended in 0.01 M phosphate buffer at pH 7 in all cases, except that the final pellet was resuspended in 0.1 ionic strength phosphate buffer at pH 6.5. The 0.01 M phosphate buffer was used because this salt concentration was found by Stanley to be optimum for virus stability. The 0.1 ionic strength phosphate buffer was used to suspend the final pellet in order to provide the proper buffer concentration for the electrophoretic studies. Further purification of the virus was obtained by electrophoretic fractionation.

Bioassay.—Cowpea (*Vigna sinensis* Endl. var. Wilt Resistant) plants were used as an assay host for measuring virus activity. The primary leaves were dusted with Celite (grade #545) to provide the necessary abrasive action and the inoculum was applied with ground-glass spatulas which had been dipped in the virus suspensions. Inoculated leaves were rinsed with water. Local lesions were counted after 4 to 6 days.

Electron microscopy.—All micrographs were taken with an RCA type EMU electron microscope. The objective pole piece of the instrument was corrected according to Hillier and Ramberg (4), and a self-biased gun was used as the electron source. Specimens for electron microscopy were prepared by the monolayer technique (3). The latter technique was developed in this laboratory primarily to improve upon existing methods of preparing specimens of biological materials, bacteria and viruses in particular, for morphological examination by light and by electron microscopy. Briefly, the technique involves the application of a suspension of virus particles in a dilute protein solution to a point on an aqueous surface. The protein spreads spontaneously to form an insoluble monolayer in which the particles to be examined are uniformly distributed. When the monolayer with its embedded particles is transferred to a solid surface, one obtains specimens that are easy to manipulate for the purpose of microscopic inspection. The specimens prepared for the electron microscope were shadowed with 8AU of uranium at a grazing angle of 11 degrees. Calibration of the microscope magnification was attained by using a replica of a precision ruled grating manufactured by the Ford Co. consisting of 15000 lines/in.

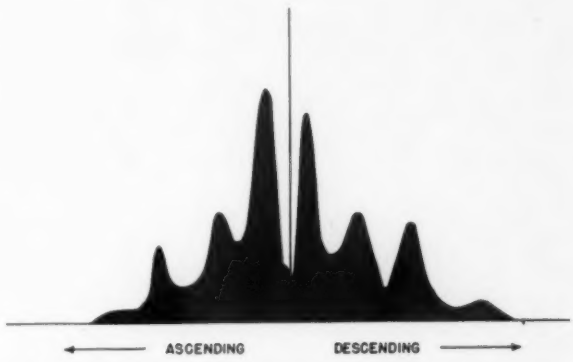

FIG. 1. Electrophoretic patterns of tobacco ringspot virus after 60 minutes at pH 6.5 in 0.1 ionic strength phosphate buffer at a potential gradient of 3.7 volts/cm. $\tau/2 = 0.38$.

EXPERIMENTAL RESULTS.—*Electrophoretic fractionation.*—Electrophoresis of preparations from infected tissue in the Tiselius apparatus revealed the presence of 4 components. The ascending and descending boundaries of these components after 60 minutes of electrophoresis are illustrated in Fig. 1, while the char-

[1] Accepted for publication August 24, 1953.

[2] Present address, Dept. of Plant Pathology, University of California, Riverside, California.

Grateful acknowledgment is extended by the authors to Drs. J. B. Bateman and J. E. Mitchell for their constructive review of this paper, to Dr. Frances Meehan Latterell for originally supplying the virus used, to Mr. J. H. Convey for the electrophoretic fractionation, to Mr. R. L. Latterell for making some of the inoculations, and to Mr. H. J. Brethauer, Jr. for preparing some of the illustrations.

TABLE 1.—*Electrophoretic mobilities of various components of tobacco ringspot virus preparation*
Virus concentration, 0.38%
Temperature, 1.3°C.
Buffer, 0.1 ionic strength phosphate, pH 6.5
Specific conductance, 1.25×10^{-4} mhos
Current, 10 ma.
Duration of run, 60 minutes
μ is $10^{-5} \times$ mobility in cm^2 $volt^{-1}$ sec^{-1}

Ascending				Descending			
Component	μ	Conc. %	Fraction Separated	Component	μ	Conc. %	Fraction Separated
1	−13.56[a]	2.0	I	1	−14.55	6.0	III
2	−10.03	14.2	I	2	−9.87	26.7	III
3	−5.45	25.7	II	3	−5.34	30.0	IV
4	−1.39	58.1	III	4	−1.18	37.3	V

[a] Estimated value.

acteristics of the components are listed in Table 1.

Following electrophoresis for 312 minutes, the material was separated into 5 fractions. The origin, infectivity, optical density and components of each fraction are shown in Tables 1 and 2.

Portions of electrophoretic fraction V were centrifuged in the Spinco Model L preparative ultracentrifuge for 7, 21 and 63 minutes at 40,000 rpm. Samples of the supernatant fluid and resuspended pellet for each time interval were prepared for examination by the electron microscope.

Electrophoresis of the healthy preparation demonstrated that only 2 components (mobilities of 14.63×10^{-5} and 10.33×10^{-5} cm^2 $volt^{-1}$ sec^{-1} respectively) were present. These values compared favorably with the mobilities of the 2 fastest moving components (1 and 2, descending boundaries, Table 1) of preparations from infected tissues.

Comparison of healthy and diseased preparations.—The preparations resulting from the differential centrifugation of juice from both diseased and healthy plant tissues were tested for virus activity. That from healthy tissue possessed no virus activity whereas that from diseased tissue had a high infectivity titer.

The ultraviolet absorption spectra of the 2 preparations were also compared. The preparation from virus infected material showed a more pronounced maximum at 260 mμ than did the preparation from healthy material.

Correlation of virus activity with particle sedimentation.—Infectivities of the supernatant fluid and resuspended pellet from each of the time periods mentioned previously were bioassayed on cowpea as previously described. Particle counts were made from 25 electron micrographs of preparations prepared by a standard procedure from the supernatant fluid and of the resuspended pellet for each time period. The particles counted were similar to those in Fig. 2. The results in Table 3 show that centrifuging for 63 minutes increased the particle count in the pellet about 4 times and decreased that in the supernatant about 17 times in relation to the corresponding values after 7 minutes centrifuging. A correlation coefficient of 0.97 for infectivities and particle counts was obtained for both the ascending and descending order. By means of the Z transformation it was shown that with 2 degrees of freedom the value of 0.97 obtained was significant at the 5 per cent level.

Particle size and shape.—Electron micrographs of the infective preparations indicate that the particles are 5- or 6-sided with a cross section measuring 22 mμ in a direction perpendicular to the long axis of the shadow. However, the shape approximates a sphere since the depth of the particles as determined by shadow measurements corresponded to a diameter of 20.9 mμ. It is to be recognized that the measurement of shadows cast by individual particles is subject to uncertainty due to local shadow angles and to the difficulty in making a precise determination of filament

TABLE 2.—*Infectivity and optical density of electrophoretic fractions of tobacco ringspot virus*

Fraction	Components present in fraction	Aver. No. Lesions per leaf[a]	Optical Density at 260 mμ.
I	1, 2	9.5	0.0205
II	1, 2, 3	33.1	0.0570
III	1, 2, 3, 4	319.9	0.0880
IV	3, 4	373.9	0.0870
V	4	301.7	0.0720

[a] Ten leaves used in each test.

TABLE 3.—*Infectivity and particle count comparison during sedimentation of Fraction V*

Duration of centrifuging (minutes)	Total number of lesions[a]		Average Electron Microscope Particle count[b] per observed area	
	Pellet	Supernatant	Pellet	Supernatant
7	3227	9170	123	371
21	6543	4052	268	243
63	8670	182	485	22

[a] 20 plants inoculated for each time period.
[b] 25 areas counted each with an area of 3.6×10^{-5} cm^2.

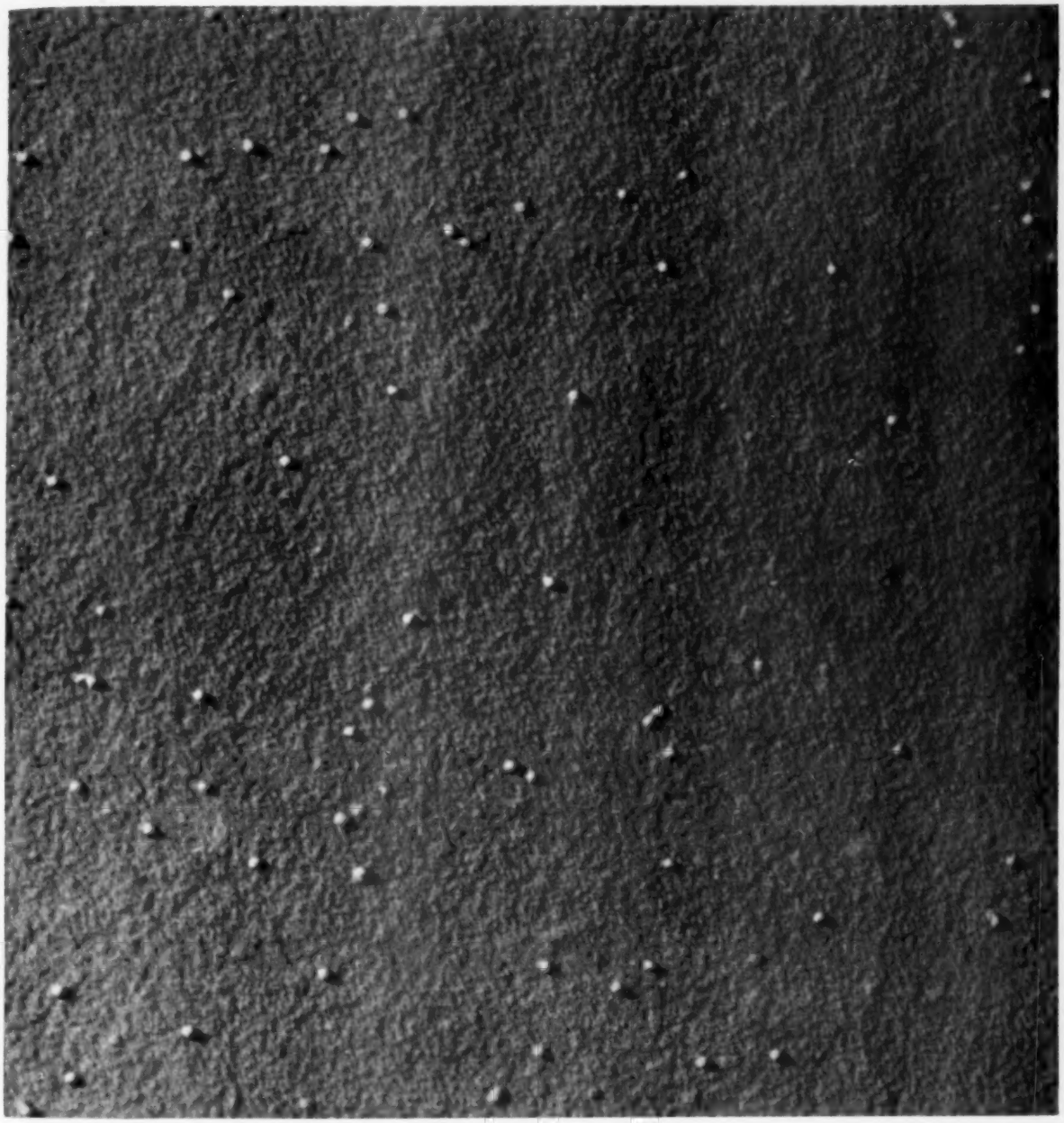

FIG. 2. Tobacco ringspot virus particles (electrophoretic fraction V) incorporated in a protein monolayer. Representative field. Uranium shadowed at a grazing angle of 11 degrees. ×27500.

position, etc. Nevertheless, it is probable that there is relatively little, if any, flattening of these almost spherical particles when the monolayer technique is employed because of the large decrease in surface tension which results from the addition of protein to the sample and to the extremely rapid drying of the monolayer on the specimen screen.

Table 4 gives results of statistical examination of particle diameter and Fig. 3 gives particle size distribution based on the measurement of 100 particles.

The preparations from healthy tissue were found to contain a few particles spherical in appearance ranging from 20 to 40 mμ with average diameter of 34 mμ and an infinite number of particles about 10 mμ.

DISCUSSION.—The figure of 22 mμ representing the average particle size of the tobacco ringspot virus purified by means of ultracentrifugation was obtained by a measuring microscope. The monolayer technique previously described results in a discrete distribution with a minimum of clumping and enables one to measure individual particles readily. The only other published electron micrograph known to the authors (2) indi-

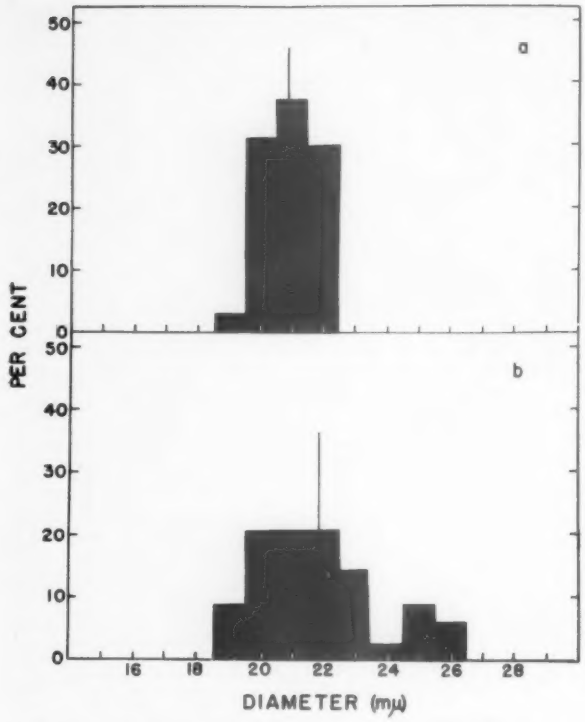

FIG. 3. Histogram showing particle size distribution of tobacco ringspot virus based on the measurement of 100 particles.

cates a diameter of 26 mμ but measurements were made from unpurified virus preparations.

Ultrafiltration studies (6, 9, 10) indicate that the particle size varies between 13-21 mμ. Ultracentrifugation studies reported by Stanley (6) indicate a size of 19 mμ. The results reported herein, i.e. 22 mμ, for the particle diameter, are in good agreement with the aforementioned ultracentrifuge data and with the upper limits of the ultrafiltration values as obtained by other workers.

TABLE 4.—*Analysis of variance of measured virus particle diameter*

	Shadow	Vertical to Shadow
Number of particles counted, n	100	100
Average diameter of particles, d	20.9	21.7
Standard deviation, s	0.69	1.94
Coefficient of variation, cv	3.3%	8.94%
Standard deviation of average, sx	0.07	0.19

Definitions: $s = \sqrt{x^2/n\text{-}1}$, where x is the deviation from the mean; $cv = 100\ s/d$; $sx = s/\sqrt{n}$.

Pirie (5) has isolated protein from healthy tobacco plants which contains 2 components with electrophoretic mobilities of 15 and 11 $\times 10^{-5}$ $cm^2\ sec^{-1}\ v^{-1}$. Of those present in the tobacco ringspot virus preparation, the 2 fastest moving components (1 and 2, descending boundaries, Table 1) had mobilities of 14.55 and 9.87 $\times 10^{-5}$ which were close to the values found by Pirie. The fraction I containing these 2 boundaries on the descending side showed the lowest infectivity of any of the fractions separated (Table 2). It is possible that the material isolated from healthy tobacco plants in these studies and also the 2 fastest moving components of the virus preparation are similar to the normal protein described by Pirie (5).

SUMMARY

Tobacco ringspot virus was obtained in a high state of purity by differential centrifugation followed by electrophoretic fractionation. Electron microscope studies of these preparations revealed the average particle diameter to be 22 mμ. The correlation coefficient between local lesion assay on cowpea plants and particle counts of electron micrographs of the resuspended pellets and supernatant fluids was 0.97, a figure which was significant at the 5 per cent level. Thus, infectivity appeared to be associated with the type of particle observed and counted.

CAMP DETRICK
FREDERICK, MARYLAND

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TRANSMISSION OF PHONY DISEASE VIRUS FROM TOPS OF CERTAIN AFFECTED PEACH AND PLUM TREES [1]

Lee M. Hutchins,[2] L. C. Cochran,[2] W. F. Turner,[3] and J. H. Weinberger [2]

Phony disease of peach and other stone fruits has been classed apart from other fruit-tree and woody-plant virus diseases because the early investigations indicated localization of the causal virus in the root wood of diseased trees (2, 3). Under the conditions and with the techniques employed in early work (2), which was largely confined to peach, no transmission of the disease was obtained with grafts employing scions, buds, or bark from affected trees. However, the disease was uniformly transmitted when roots or piece-root sections containing living wood from diseased trees were grafted onto the roots of normal trees. New evidence now indicates that transmission can be effected with scions from at least parts of the tops of certain phony-affected peach and plum trees. Failure to transmit from the tops of some phony trees and transmission from only certain arms of others when similar graft inoculation techniques were used indicate that the phony virus is not uniformly present in the tissues of all affected trees.

PASSAGE OF PHONY VIRUS DOWNWARD THROUGH STEMS.—The ability of phony virus to pass downward through peach stem tissue was first indicated in an experiment performed in the early 1930's; in this the tops of several small healthy nursery peach trees, growing in boxes on raised platforms, were grafted to the roots of small trees made phony by root-graft inoculations. The nursery trees were severed from the phony trees after 2 growing seasons and transplanted to a nursery row. In all cases where a successful graft union formed, the nursery tree developed typical phony, indicating that the causal virus had passed downward through the graft union and the stem to the roots of the nursery tree.

In another series, transmission of the virus downward through stems was accomplished where pieces of roots from phony-affected peach trees were grafted at varying heights up to 14 in. above the ground line onto the trunks of healthy 4-year-old peach trees. Again, many of the nursery trees thus inoculated developed typical phony, indicating infection through the stem tissues.

PHONY VIRUS IN TOPS OF PLUM TREES.—As early as 1937, evidence which indicated that phony virus was present in the tops of affected plum species was obtained. In 1935 a transmission series was set up in which scions from an experimentally phony-infected chickasaw plum (*Prunus angustifolia* Marsh.) tree (infected by root-graft inoculation from a phony-affected peach) were used as follows: 1) crown grafts on peach roots (diseased plum scions were bench whip-grafted onto the crowns of healthy decapitated peach seedlings); 2) piece-root grafts on hortulan plum (*P. hortulana* Bailey) roots (diseased plum scions were bench whip-grafted onto pieces of healthy hortulan plum roots). Also stem cuttings were rooted from the same tree. Phony-appearing trees developed in all the lots. The peach roots of affected trees gave a positive reaction to the chemical test for phony disease, developed by Hutchins (2). In subsequent series where scions taken from experimentally infected phony trees of chickasaw plum, Mexican plum (*P. mexicana* S. Wats.), and hortulan plum (infected by root-graft inoculations from phony-affected peach) were whip-grafted at the ground level onto decapitated healthy peach seedlings, a high percentage of the resulting plum trees developed phony symptoms. These trees demonstrated that the virus can invade the tops of phony-infected plum trees.

Additional evidence was obtained on the occurrence of phony virus in the tops of chickasaw plum in experiments made in the summer of 1944. Forty-three plum trees were dug from a natural thicket and transplanted to the nursery row; all were tested by grafting root-pieces onto peach and 17 of the plums proved to be phony-infected. Scions were cut from 14 of the plum trees which appeared dwarfed, including 11 of the 17 that were proved diseased, and were grafted onto the tops of peach seedlings. Transmission resulted from all 11 plum trees. This high percentage of transmission with plum scions indicates that the phony virus may more generally invade plum than peach tops (Table 1).

PHONY VIRUS IN TOPS OF PEACH TREES.—Proof that phony virus was able to exist in the tops of plums and to pass downward in peach stems to the roots made it necessary to re-examine experimental procedures and results relative to the virus-tissue relations in peach. In the spring of 1939, in the experimental nursery at Fort Valley, Georgia, scions of phony Uneeda, Hiley, and Augbert peaches and of a seedling peach were whip-grafted onto decapitated healthy peach seedlings at the ground level and the scions developed into trees. Of the 24 trees resulting from growth of the scions, 17 were visibly phony by August 1940, indicating that the phony virus was present in the tops of the peach trees from which the scions were cut. No phony trees developed in parallel check series growing in the

[1] Accepted for publication August 25, 1953.

Credit is due the late John L. Rue, who assisted in planning and setting up much of this work, and Mrs. J. L. Rue for assistance in arranging and interpreting records. Appreciation is expressed to Glenn KenKnight for criticisms and suggestions during preparation of this paper.

[2] Respectively, Head Pathologist, Principal Pathologist, and Senior Horticulturist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[3] Entomologist, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

TABLE 1.—*Top transmission of phony virus to Hiley peach, 1942 summer series, Fort Valley, Georgia, by graft inoculations made July 5, 1942*

Source of inoculum	Type of inoculation	Number of trees in treatment	Number with living inoculum Aug. 25, 1942	Number of trees phony July 1944	Number of trees living 1945	Number of trees phony July 1945
Uneeda peach, Lot 175-39-T22	2 straight scions	10	10	7	10	8
Do	2 heel-sprurs, 1942 growth	6	6	3	5	5
Do	2 stalked buds	12	12	1	10	5
Do	2 shields—bark only	5	5	0	3	2[a]
Do	2 heel-spurs, 1941 heel, 1942 spur	10	10	4	8	8
Elberta peach	2 straight scions	10	9	0	10	1
Do	2 heel-spurs, 1942 growth	7	7	0	7	0
Do	2 stalked buds	10	10	0	10	1
Do	2 heel-spurs, 1941 heel, 1942 spur	10	10	0	10	0
Do	2 shields—bark only	10	10	0	10	0
Chickasaw plum	2 straight scions	10	6	3	10	4
Do	2 heel-spurs, 1941 heel, 1942 spur	10	10	8	10	10
Do	2 stalked buds	10	10	4	10	10
Do	2 shields—bark only	10	10	0	10	3
Do	2 root-to-root	5	—	5	5	5
Uneeda peach, Lot 175-39-T22	2 root-to-root	5	—	5	5	5
Checks	None	137	—	0	137	6[b]

[a] Natural spread, not result of grafts.
[b] Represents natural spread.

nursery alongside the grafted seedlings.

Transmission from peach tops.—In the spring of 1942, a series of inoculations, hereafter called the 1942 dormant season series, was made by bench whip-grafting dormant phony peach scions onto the crowns of decapitated nursery trees of Hiley peach and then planted in nursery rows. The inoculum was taken from typically phony trees developed from diseased scions in Lots 175, 177a, and 177 in the nursery mentioned in the preceding paragraph and from naturally infected orchard trees. In addition, 1 lot of 5 Hiley peach nursery trees was root-to-root inoculated from each of the phony source trees and the respective inoculated lots were separated by 5-tree noninoculated lots as checks. By July 1943 many of the trees grown from the phony scions appeared phony. In August the trees were lifted and all were subjected to the chemical test for phony. Transmission resulted with scions from 12 of the 13 phony source trees (Table 2). No cases of phony developed in the untreated checks. Trees propagated from Lots 175, 177a, and 177 represent serial perpetuation, but transmission from 5 of the 6 naturally infected orchard trees is indicative that the virus may be common in the tops of some naturally infected orchard trees.

In July 1942 another series of inoculations, hereafter called the 1942 summer series, was made during the growing season on Hiley peach nursery trees, to parallel the one made with dormant scions the previous March. Short straight scions inserted in T-cuts and a new type, termed heel-spur scions (a bark and wood shield taken at a branch node so that in place of a dormant bud it bore a spur or short piece of shoot), were used. A heel-spur scion can be used by inserting the shield in a T-cut similar to that made for budding at any time during the growing season when the bark will slip. Observations indicate that the wood portion in such scions remains alive in a higher percentage of cases than it does in straight scions. A third type of scion inoculum called stalked buds (bark and wood shield bearing a short spur, $\frac{3}{8}$-$\frac{3}{4}$ in. long, with a terminal bud at the apex) was also used. This last is essentially a heel-spur type, but it is occasionally used by nurserymen in dormant budding. In the 1942 summer series there were 3 sources of inoculum: 1) a phony Uneeda peach tree produced by growth from a diseased scion, Lot 175-39-T22, the same as used in the dormant season series; 2) a phony 4-year-old Elberta peach tree, naturally infected and showing symptoms for the first time in 1942, which gave a positive chemical test for phony and remained phony until removed in 1943; and 3) a phony chickasaw plum tree, naturally infected, which had been transplanted to the nursery row from a thicket. (This last tree was dwarfed as compared with others and had been proved phony by transmission to peach through root-to-root grafts.) Untreated check lots were left between the respective inoculated lots. Two groups of 5 peach trees each were root-to-root inoculated, one group from Lot 175-39-T22 (peach) and the other from phony

TABLE 2.—*Scion transmission of phony virus, 1942 dormant season series, Fort Valley, Georgia*

Source of inoculum	Mode of infection of inoculum source trees	Number of grafts made March 1942	Number survived	Number phony[a] August 1943
A. Scions from phony trees bench whip-grafted onto crowns of decapitated Hiley peach nursery trees:				
Uneeda peach—Lot 175-39-T9	Diseased scion	20	12	10
Do —Lot 175-39-T13	do	20	4	4
Do —Lot 175-39-T22	do	40	7	7
Do —Lot 175-39-T10	do	20	3	3
Do —Lot 175-39-T17	do	20	4	4
Hiley peach—Lot 177a-39-T2	do	40	8	8
Seedling peach—Lot 177-39-T1	do	20	12	9
Elberta peach—DA Orchard	Natural	41	10	9
Do —C Orchard-T1	do	21	15	15
Do —C Orchard-T2	do	21	10	7
Do —C Orchard-T3	do	24	13	12
Do —C Orchard-T4	do	19	2	0
Do —C Orchard-T5	do	23	11	11
Total		329	111	99
B. 15 index lots (5 Hiley peach nursery trees each) grafted with roots, 1 lot from each phony source tree[b]		75	61[c]	61
C. 15 check lots (5 Hiley nursery trees each) of noninoculated trees		0	75	0

[a] Readings based on visual symptoms and positive reaction to the chemical test for phony.
[b] 2 of 15 sources were not recorded under "A" because of failure of scions to live.
[c] Number recorded was number on which union with phony root was successful.

chickasaw plum. Results are given in Table 2.

By July 1944 transmission had resulted with both current-season and older wood from the Uneeda peach and the chickasaw plum, but no transmission had resulted from the Elberta peach. Since the grafts were made under comparable conditions, failure to transmit from the Elberta peach indicates that the virus was not present in the top tissues from which the scions were cut. The increase in percentage of infection in some lots in 1945 indicates a 3-year incubation in some of the trees; about 4 per cent of the increase, including the 2 cases from the Elberta tree and the 2 cases from the bark only from the Uneeda tree, must be credited to infection through natural spread, as shown by the percentage of disease in the noninoculated checks. Symptoms on the root-inoculated series developed earlier, and were persistently more pronounced than on any of the scion-inoculated trees throughout the tenure of the experiment.

To investigate further the differential occurrence of the phony virus in the tops of phony-affected peach as possibly affected by variety and by the length of time trees had been diseased, 2 additional series were set up in 1944 and 1945 at Brownwood, Texas.

The first of these, set up in the spring of 1944, involved piece-root grafts (phony scions bench whip-grafted onto small pieces of healthy peach roots), crown grafts (phony scions bench whip-grafted at the crown onto decapitated peach seedlings), whip-grafts on 2-year-old growing trees (phony scions whip-grafted 3–4 ft. above the ground level onto trees just breaking dormancy), and root-to-root grafts. The inoculum was taken from 6 naturally infected trees showing pronounced phony symptoms in a 10-year-old Elberta peach orchard near Marshallville, Georgia. Wet weather at planting time caused much of the material to be lost and survival could be recorded on only 3 combinations in August 1946 (Table 3).

In the 1944 dormant season series only 3 of 51 trees resulting from the bench grafts became phony, whereas 21 of the 61 trees inoculated from the same sources by whip-grafting diseased scions onto branches of nursery trees became phony. In the latter series the inoculated trees were not removed from the ground, whereas in the former the trees were lifted and replanted after grafting. Presumably the virus was equivalent in the scion material used in both cases; therefore other factors are responsible for the increased transmission in the whip-graft series. It is logical that scions whip-grafted directly onto a growing tree would make a quicker union than scions on a tree that had been lifted. It therefore seems reasonable that the speed of the union may be an important feature in transmission of the phony virus; perhaps the phony-bearing tissue of the scion may die before the virus is translocated to the stock unless a living wood bridge is formed rapidly.

In the second series, initiated in August 1945, inoculum was selected from 21 naturally infected Elberta peach trees currently showing symptoms for the first time and from 32 naturally infected trees of 4 varieties

TABLE 3.—*Top transmission of phony virus from dormant Elberta peach scions, February 1944 series, Brownwood, Texas*[a]

Phony source tree No.	Decapitated nursery trees bench whip-grafted on the crown before planting			2-year-old Elberta nursery trees scion whip-grafted while breaking dormancy		Elberta nursery trees root-graft inoculated before planting	
	Number from each source tree	Number on which scion grew	Number becoming phony	Number from each source tree	Number becoming phony	Number from each source tree	Number becoming phony
1	25	1	0	10	5	5	5
2	25	13	0	10	0	5	5
3	25	8	0	10	2	5	5
4	25	0	0	11	5	5	5
5	25	19	1	10	6	5	5
6	25	10	2	10	3	5	5

[a] Readings were made in August 1946. Checks composed of 7 lots of 5 trees each top grafted with scions from known healthy trees, interspersed among the other series, remained healthy.

(8 trees each), Hiley, Elberta, Sullivan Elberta, and Uneeda, which had been diseased 3 years or longer. The scion wood was collected from the diseased trees in the vicinity of Fort Valley and Marshallville, Georgia, packed in damp peat moss and shipped via air express to Brownwood, Texas, where it was grafted 2 days later onto 4-year-old J. H. Hale peach trees. Five trees were each inoculated with 2 heel-spur scions and 1 tree was root-graft inoculated from each source tree. Appropriate untreated checks were left at regular intervals. Results are given in Table 4.

Only 3 of the 50 source trees in the 1945 Brownwood summer series showed transmission after a 2-year incubation period. Twenty-one trees, representing 9 of the 50 source trees, were phony 3 years after inoculation and 2 additional trees in one of the series became phony after 4 years' incubation. No phony occurred in any of the 95 untreated checks, indicating that none of the cases was a result of natural spread. The inoculations were made with heel-spur scions at approximately 6 ft. above the ground level in 4-year-old trees. It thus appears that where inoculations are made high in a tree or in larger trees the incubation period is longer than the 18 months indicated for small nursery trees.

Further evidence that phony virus can effect symptoms in tops of peach trees without being present in tissues.—In addition to scion grafting, Hutchins (2) attempted transmission of phony disease by grafting young healthy nursery peach trees by their taproots to the tops of typically phony, naturally infected, large peach trees. Early in the growing season, while they were attached to the phony tree, the roots of the nursery tree, the graft union, and a piece of the phony tree stem were encased in a soil-filled pail. A good functional union was evidenced by the fact that the nursery trees lived thus attached to the phony tree during an entire growing season and became typically phony in appearance. In January, 12 months after the grafts were made, the nursery trees were severed at the base of the pail so that a piece of the stem of the phony tree remained attached; they were then removed from the pail and planted in the nursery row. During the first growing season after transplanting, all the trees outgrew the phony condition and remained normal in appearance for several years.

TABLE 4.—*Top transmission of phony virus from peach trees with different ages of symptom expression to J. H. Hale peach, August 1945, Brownwood, Texas*

Phony source variety	Age of symptom expression	Number of phony source trees—				
		Indexed	Transmitting through root grafts	From which heel-spur scions became established in peach nursery trees	Transmitting from tops 1947	1948[a]
Elberta	current season	21	15	21	2	5
Hiley	3 years or more	8	6	8	0	1
Elberta	do	8	6	8	0	2
Sullivan Elberta	do	8	4	7	0	0
Uneeda	do	8	6	6	1	1
Total	---	53	37[b]	50	3[c]	9
Untreated checks	---	---	---	95[d]	0	0[e]

[a] All the trees which were diseased in 1947 and 1948 showed symptoms in 1949 and 2 additional ones were diseased.
[b] Failure to transmit with root grafts from some of the phony source trees is due to failure of the root pieces to make growth union.
[c] Transmission in all 3 cases was represented by a single tree with the other 4 of the lot healthy.
[d] Randomized in lots of 5 between the treated lots.
[e] All normal in 1949.

In light of evidence that plant viruses generally move rapidly from tops to roots and slowly in the reverse direction, it could be argued that the phony virus might have been present in the tops of the phony tree and that the nursery tree was not attached long enough for movement of the virus into it. Such a hypothesis does not explain the phony symptoms that developed in the nursery trees while they were attached or what happened to the virus if it was present in the stem piece of the phony tree that remained attached to the nursery tree root when it was transplanted.

Based on information indicating that the phony virus moves downward to roots readily when introduced into peach tops, a new series of experiments was made in Brownwood, Texas. Peach seedlings were planted in the ground under phony trees and were inarched into individual scaffold arms of the trees. When the unions were well developed, the arms of the phony trees were girdled on the trunk side of the inarch and the phony arms were thus transposed to a new root system. Inarches were made on approximately 60 phony trees, 4 seedlings per tree, in May 1943. The arms were girdled on 30 trees on February 4, 1944, and successful transfer of arms to 83 seedlings resulted. Arms on another 20 trees were girdled on October 6, 1944, and transfer of arms to 55 seedlings resulted. The portion of the original phony tree died on some of the seedlings during the summer of 1944 and winter of 1944-45, but on many other seedlings it was alive and put out shoots during the 1945 growing season. All arms of the originally phony trees successfully transferred to the seedlings and thus to a new root system developed apparently normal vigorous growth, whereas those on the 10 trees to which the seedlings remained inarched and which were not severed from their original roots continued to be phony. Some of the transposed arms were still standing in 1948 and were normal in growth habit, indicating that the virus was not present in them. This agrees with earlier research by Hutchins (2), who concluded that phony symptoms could appear in the tops of the trees even though the virus was present only in the roots.

Discussion.—Contrary to the early report that in peach the phony virus appeared to be limited to the roots of diseased trees (2), transmission has been repeatedly obtained with scions from the tops of diseased peach and plum trees. Results appear to indicate that generally the virus is not uniformly distributed throughout the tops of affected trees, and that success even with scions containing the virus is dependent upon a suitable union.

The earlier conclusion that the phony virus was not present in the tops of affected peach trees was based upon failure to transmit from them, when various combinations of budding and grafting were used, whereas any combination involving roots from phony trees as inoculum regularly transmitted the disease. The majority of the early transmission trials with scions were piece-root grafts (a small piece of healthy root whip-grafted with a diseased scion). Piece-root grafts of peach are notably slow to start and examination of scions after growth had started showed the center of the woody cylinder discolored and apparently dead. It is plausible that failure of transmission by such combination is due to death of the virus or virus-bearing tissue of the scion before the virus was translocated to the stock. The early work was done at Fort Valley, Georgia, where natural spread occurred in the test plots; thus a low percentage of transmission may have been obscured, particularly if no symptoms developed until the third year after inoculation.

In the work reported here, which resulted in transmission from the tops, scions from diseased trees were grafted onto peach as follows: bench whip-grafted onto decapitated seedlings and planted immediately, whip-grafted onto decapitated seedlings without removal from the ground, whip-grafted onto the tops of nursery trees in the spring just prior to the beginning of growth, or grafted by inserting scions under the bark in T-cuts during the growing season. In all of these combinations, the graft union develops more rapidly than on piece-root grafts.

The apparent uneven distribution of the phony virus in the tops of naturally infected phony peach trees is difficult to explain. Hutchins (3) showed that in roots the virus was limited to the woody cylinder; it seems probable that it is likewise limited in stems. Esau (1) showed that the woody cylinder of peach roots contains much more living tissue than that of stems and, further, the living tissue in roots is more extensively interconnected than that in stems. Esau found no morphological peculiarities that would prevent virus movement from roots to stems or throughout stems. Logically, with more space and better interconnected living tissue, a larger volume of virus develops and becomes better distributed in roots than in stems. At any rate, phony has been transmitted regularly with root inoculum and erratically with scions. Where phony trees were produced by growth from a diseased scion, it is evident that the virus was present in the diseased scion. Consistent transmission from both roots and tops of such trees indicates that the virus is well distributed in them. It further indicates that when the virus invades tissues of the stem growing point it becomes more thoroughly distributed in the subsequent top growth, and once in the tissues some of it remains there.

Determination of the presence of the phony virus is difficult and has been done solely on the basis of graft transmission. Failure of transmission with scions from a phony tree may not necessarily be proof that the virus was not in the tissues sampled; as previously suggested, the virus may be present but the portion of tissues supporting the virus may die before the virus is translocated to the stock even though the scion grows. In the pail experiment, where nursery trees grafted by their taproots to stems of phony trees failed to acquire the virus (2), the argument might still be presented that they were not attached long

enough for the virus to move upward into them. The nursery trees remained attached during one entire growing season, however, and a good union was evidenced, not only by their survival, but also by development of typical phony symptoms similar to those of the trees to which they are attached. Further, when they were severed from the phony trees, a living piece of the phony tree stem was left attached to the root; thus presumably if the virus had been present it would have infected the nursery trees.

In the transposition experiment, in which arms of pronounced phony peach trees recovered when transposed to healthy seedlings inarched to them, it is difficult to explain the recovery on any other basis than that the phony virus was not present in the transposed arms. The phony trees in the transposition experiment were infected through root grafts made 5 years prior to inarching. It thus appears that the phony virus may not move or moves very slowly from peach root to top. Correspondingly, it seems reasonable that if the virus is introduced low in the tops of peach trees it is less likely to become distributed in tissues above the inoculation point than below it. Since symptoms of the phony disease are generally uniform in tops of peach trees, selection of scions that are virus-bearing is a matter of chance, depending on the proportion of the top invaded. Movement of the phony virus from roots to tops in 3 species of plums was demonstrated by scion transmission from previously root-inoculated trees.

Kunkel (5) suggested that the absence of the phony virus in peach tree tops may be due to its inactivation by high summer temperatures. Hutchins and Rue (4) showed that a minimum of 40 minutes at 48°C. was needed to inactivate the virus in small trees. The effect of lower temperatures for longer duration such as occurs in orchards is not known. Opposed to the heat inactivation theory proposed by Kunkel is the fact that transmission was effected regularly at various times during the growing season from certain orchard trees, indicating that, once the phony virus becomes established, some of it remains there.

The length of the incubation period in peach appears to depend upon sufficient build-up of the virus in the roots. Where healthy scions were grafted onto a large piece of phony root, growth was phony from the start, whereas scions on a small piece of root required the usual 18 months for symptom expression. In the present studies, scion inoculation of small trees resulted in a shorter incubation period than similar inoculations of larger test trees, and, in general, the incubation period was longer following scion inoculations than following root inoculations. In 1 experiment (Table 4) in which 4-year-old trees, approximately 8–10 ft. in height, were scion-inoculated only 3 of 265 test trees were phony after 2 years; 27 were phony the following year; and 2 additional trees became phony in the fourth year, whereas trees of the same age infected by root grafts were all phony at the end of the second year after inoculation. It is also possible that the incubation period may be influenced by the distance between the infection point and the roots.

SUMMARY AND CONCLUSIONS

Contrary to previous results indicating that the virus that causes phony disease of peach is restricted to the roots, transmission with scions has shown that the virus can exist in the tops of affected trees. Transmission from certain affected trees and from certain arms on affected trees but not from others indicates that the causal virus is not uniformly distributed in the tops. Scions taken from trees grown from virus-bearing scions gave consistent transmission, indicating that when the virus invades the growing point it becomes more uniformly distributed in subsequent tissue and once in th etissue some of it remains there. Preliminary results indicate that the virus may not move into tops or moves very slowly into tops of peach trees inoculated in the roots.

The virus appears to be more generally distributed in the tops of affected plum trees than in those of peach.

Transmission from tops was effected with various graft combinations in which the woody cylinder of the infected scions was kept alive until new growth started. Failure to transmit from tops of trees in the past is attributed in part to the use of graft combinations in which the wood of the scion died before sufficient union was formed to permit transmission.

Symptom expression of phony appears to depend on the effect of the virus on root tissues and the length of the incubation period is correlated with build-up of the virus in the roots. Large trees scion-inoculated varied in incubation period from 2–4 years, whereas small trees usually developed good symptoms in 18–24 months. Root-inoculated peach trees consistently developed symptoms in 18 months. They showed more pronounced symptoms than scion-inoculated trees during the early stages of the disease.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

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AN IMPROVED METHOD OF INOCULATING SEED OF OATS AND BARLEY WITH SMUT [1]

W. Popp and W. J. Cherewick

In the inoculation of oat and barley seed with smuts, the presence of hulls surrounding the kernels makes it necessary to employ a technique that will enable the chlamydospores to be brought into contact with the caryopsis. This is essential in field experiments where the common method of dusting the surface of seed with spores frequently fails to result in infections heavy enough to permit determination of the relative susceptibility of oat and barley varieties.

Several methods which vary in efficiency and suitability have been developed (1, 2, 4). The technique of inoculation described in the present paper is an attempt to improve earlier methods.

DESCRIPTION OF THE METHOD.—The improved method consists essentially of violently agitating the seed while it is immersed in a water suspension of chlamydospores. A high speed electric homogenizer (Waring Blendor) is used for this purpose. The Blendor jar is the metal type and the 2 parts of the cutter knives within it are replaced with 2 blunt blades of the size and shape shown in Fig. 1. These blades tend to eliminate seed injury. They are made of 18-8 stainless steel sheet 0.094 in. in thickness and are silver-soldered together to keep them in line. All sharp corners of the blades are slightly rounded off with emery paper.

The inoculation procedure consists of placing seed in the jar of the Waring Blendor, adding the spore suspension, and agitating the seed for a period of 10–25 seconds. During the operation of the Blendor, kernels are impelled by the blades running at high speed and thus the hulls are loosened and cracked, facilitating penetration of inoculum between the hull and the caryopsis. Upon completion of agitation, the contents of the Blendor jar are poured into a 20-mesh sieve to separate the seed from the suspension. The inoculated seed is then immediately packaged into coin envelopes, and left to dry slowly for 2 days at room temperature. Slow drying of the seed permits the spores to germinate and develop a certain amount of mycelium underneath the hull. Bottoms of the envelopes are usually stapled to prevent them from coming apart while holding the wet seed.

The time for agitation of oats and barley in the spore suspension varies, depending on thickness and persistence of the hulls. Hulls of some varieties are easily loosened; duration of mixing should therefore be short to avoid seed injury. For most seed lots a period of 16–25 seconds can safely be employed but in exceptional cases a mixing time of 10 seconds may be necessary. Some seed injury, however, cannot be avoided if machine-threshed seed is used, because such seed nearly always contains some cracked hulls or dehulled kernels.

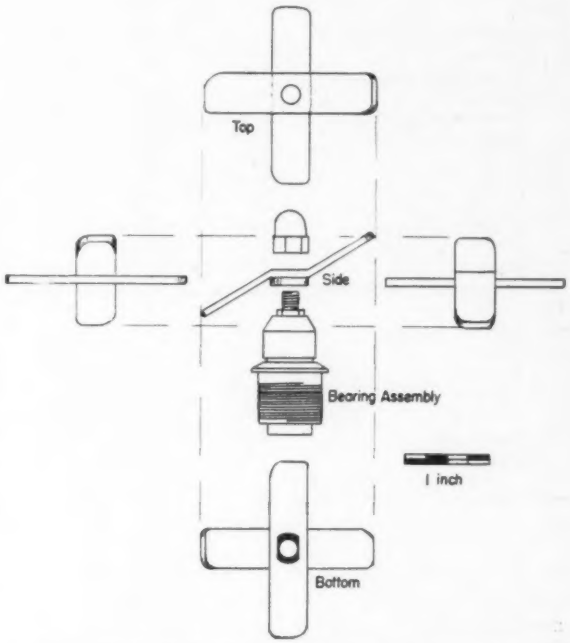

FIG. 1. Blunt blades used in place of the cutter knives of an electric homogenizer (Waring Blendor) in inoculating seed of oats and barley with smut-spore suspensions.

Concentration of the spore suspension is an important factor in the improved method of inoculation. Results of an experiment (Table 1) in which seed lots of Trebi barley and Victory oats were inoculated with spore suspensions of different concentrations showed that, for a high percentage of infection, a ratio of at least 1 gm. of spores to 1 l. of water is required.

Volume of spore suspension and size of seed lot also are important. Up to 1,000 kernels may safely be inoculated in the Blendor if a sufficient volume of spore suspension is used. For a seed lot of about 300–400 kernels, approximately 200 ml. of suspension,

TABLE 1.—*The percentage covered-smut infection obtained with Trebi barley and Victory oats by using spore suspensions of different concentrations for inoculation by the Waring-Blendor method*

Concentration of spores in suspension	Percentage of smutted heads	
	Barley	Oats
1 gm. in 200 ml. of water	23.7	36.9
1 gm. in 1000 ml. of water	21.1	32.8
1 gm. in 5000 ml. of water	14.4	21.5
1 gm. in 25000 ml. of water	9.4	15.0

[1] Accepted for publication August 28, 1953.
Contribution No. 1296 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

TABLE 2.—*The percentage covered-smut infection obtained in different years by inoculating seed of barley and oats by different methods*

Grain and variety	Year tested	Method of inoculation and percentage of smutted heads: Waring Blendor	Dry spore (dehulled seed)	Spore suspension	Dry spore (normal seed)
Barley					
Hannchen	1948	20.3	22.9	11.3	6.5
Hannchen	1949	33.3	33.0	8.8	2.9
Hannchen	1950	56.5	58.6	20.2	4.0
Hannchen	1951	26.6	23.9	11.2	1.3
Hannchen	1952	30.0	32.6	7.9	1.8
Trebi	1948	28.2	26.6	11.3	5.4
Trebi	1950	41.1	37.2	25.6	7.1
Trebi	1951	25.8	22.9	17.2	2.0
Trebi	1952	21.6	14.8	7.3	2.8
Plush	1950	79.2	76.8	40.9	15.9
Plush	1951	48.6	45.2	33.9	8.9
Odessa	1950	63.3	67.5	31.9	9.2
Average percentage		33.6	32.9	17.3	4.6
Oats					
Gopher	1949	71.8	68.1	37.7	4.8
Gopher	1950	32.4	35.4	14.0	5.3
Gopher	1952	59.8	26.0	18.5	4.0
Victory	1951	32.2	30.6	12.9	3.6
Victory	1952	64.2	46.1	35.6	8.0
Vanguard	1950	41.1	38.0	24.3	5.4
Anthony	1951	72.0	71.2	58.1	9.3
Average percentage		52.1	44.0	28.8	5.5

or just enough to cover the mixing blades, is required. For larger lots, ranging up to 1,000 kernels, a volume of 300 to 400 ml. may be necessary. When a series of seed lots is being inoculated, the suspension is kept at a constant level in the Blendor by replenishing the amount used up by each lot of seed.

To avoid the lengthy procedure of grinding smutted heads in a mortar and sifting the spores through a fine mesh sieve, a method of preparing the spore suspension in the Waring Blendor was devised. In this technique a dense spore suspension is prepared by placing smutted heads in the Blendor jar, adding distilled water, and operating the Blendor for about 1 minute. The resulting suspension is poured through an 80-mesh sieve to free it of the coarser debris. Distilled water is then mixed with the suspension until it matches the turbidity of a standard containing 2 gm. of smut spores per litre of water. The suspensions are examined and compared with one another in 1-inch test tubes while being held up to the light. If the standard suspension contains 0.1 per cent of mercuric chloride its original color is preserved and it may be used over a period of at least several months.

INOCULATION STUDIES.—The spore-suspension method of Tapke and Bever (2) and the method involving the dusting of normal and dehulled seed with dry spores were used as checks in studying the effectiveness of the Waring-Blendor method. This was essential, since, as shown by Tapke (3), the factors governing smut infection will to a great extent determine the degree of infection in plants. If conditions are favorable heavy infection may result from all methods of inoculation (3), and it may not be possible to draw conclusions as to the relative effectiveness of different methods. If, however, the environment is less favorable for infection a light infection only will result from an inferior method.

To control possible infection resulting from seed-borne smut spores, dehulled seed was treated for ½ hour and normal seed for 1 hour with formaldehyde solution (1-320). The seed was then washed in running water for ½ hour and spread out to dry. For inoculation by the Waring-Blendor method and the spore-suspension method a spore concentration of 2 gm. per litre of distilled water was used. Seed was agitated for 25 seconds in the Waring-Blendor method. For inoculation of normal and dehulled seed by the dry spore method, spores were applied at the rate of 1 gm. to 500 gm. of seed. The seed was sown with a V-belt seeder in rows 6 ft. long, 1 ft. apart, and replicated 3 times. Results of inoculations of barley and oats with covered smut (*Ustilago hordei* (Pers.) Lagerh. and *U. kolleri* Wille, respectively) are in Table 2.

The results (Table 2) of the different methods of inoculation with covered smut of barley and of oats showed relatively high percentages of smutted heads for the Waring-Blendor and the dehulling methods and both methods tended to be equally effective except in 1952 when the former resulted in a considerably higher percentage of smut in Trebi barley and in Gopher and Victory oats. The reason for this unusual variation has not been determined. Percentages of infection resulting from inoculation of seed by the spore-suspension technique were relatively low but not so low as those resulting from the inoculation of undehulled (normal) seed with dry spores.

No results have been tabulated for percentages of

infection in barley and oats inoculated with *U. nigra* Tapke and *U. avenae* (Pers.) Rostr. respectively. In 1 experiment, results very similar to those shown for *U. hordei* and *U. kolleri* were obtained.

The Waring-Blendor method saved time and labor in these studies. It is well adapted to the inoculation of a large number of seed lots or varieties of grain. In smut race identification studies made at Winnipeg 2 persons have inoculated and packaged about 90 to 100 seed lots per hour.

SUMMARY

A method is described for inoculation of barley and oat seed with water suspensions of smut spores by the use of a high speed electric homogenizer (Waring Blendor). It has proved more effective than the spore-suspension technique and as effective as that of dehulling seed and dusting it with dry spores, and is well adapted to studies in which a large number of seed lots or varieties of grain are to be inoculated.

LABORATORY OF PLANT PATHOLOGY,
WINNIPEG, MANITOBA, CANADA

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PHYTOPATHOLOGICAL NOTES

Sterile Conks of Fomes igniarius on Aspen. S. B. SILVERBORG. Sterile conks of *Fomes igniarius* (L. ex Fries) Kickx. on hardwoods have been reported by several investigators in the past. In 1936 Verrall [1] reported sterile, rimose nodules on white and yellow birch in Minnesota that he identified as *F. igniarius*. His identification was based on cultural similarity to *F. igniarius* and by the fact that both sterile nodules and normal sporophores occurred together on birches that had died recently. Weir [2] in 1915 found abortive fruiting bodies on alder which he concluded to be *F. igniarius* because of their proximity to typical fertile sporophores of *F. igniarius*, and because both fertile and sterile forms appeared to originate from the same core of diseased wood. Weir compared the abortive forms from alder and the sterile conks commonly occurring on birch and concluded that both were abnormal forms of *F. igniarius*. Mme. Katayevskaya [3] in 1928 made comparative cultural studies of *F. igniarius* and of "peculiar black fungous excrescences" occurring on stems of birches in Siberia. She tentatively identified the unknown fungus as a sterile form of *F. igniarius* although it differed markedly in appearance and microscopic characteristics from her known isolates of *F. igniarius*.

In 1938 Campbell and Davidson [4] reported a brown *Poria* associated with sterile conks on dead birch snags. Cultures from both the sterile conk tissue and the *Poria* were identical. Dr. L. O. Overholts identified the fungus and placed it in the *Poria obliqua* (Pers. ex Fries) Karst. complex.

The sterile conks described by the early investigators were not proven conclusively to be *F. igniarius*. In view of the findings of Campbell and Davidson the sterile fungus which the early workers designated as *F. igniarius* may have been *P. obliqua*.

During the summer of 1952 the writer found 3 sterile conks on a recently-killed, standing quaking aspen (*Populus tremuloides* Michx.) at the State University of New York, Huntington Wildlife Forest, Newcomb, N. Y. They were growing at a branch node located 10 ft. from the ground on a tree 57 ft. high and 8 in. d.b.h. The conks (Fig. 1, A) were black, rimose, obtuse-elongate growths 1–1½ in. in diameter at the base and protruded approximately 2 in. from the trunk. They are similar to sterile conks of *Polyporus glomeratus* Peck, common on beech (*Fagus grandifolia* Ehrh.) in the Northeast. The white heartrot (Fig. 1, B) associated with the conks was typical of *F. igniarius* rot in aspen. The decay extended for a distance of 4½ ft. above and below the conks.

Tissue cultures were made on 2 per cent malt agar from sterile conk material and from decayed wood. Cultures from both sources were identical. A strong wintergreen odor, characteristic of *F. igniarius* isolates from aspen, was produced by the cultures from both sterile conk tissue and from decayed wood. The fungus was identified as *F. igniarius* by cultural characteristics [5] and by comparison with known isolates of *F. igniarius* from quaking aspen.

This is believed to be the first report on the occurrence of sterile conks of *F. igniarius* authenticated by

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[2] Weir, J. R. 1915. Some observations on abortive sporophores of wood-destroying fungi. Phytopathology 5: 48-50.

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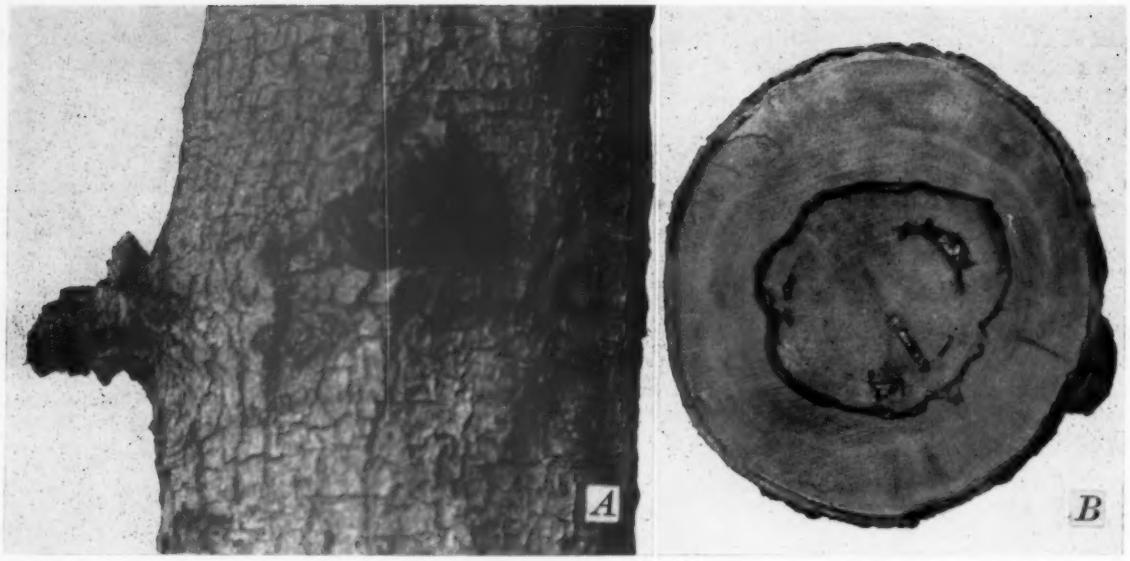

FIG. 1. A) Sterile conks of *Fomes igniarius* growing at branch node of a recently-killed quaking aspen. B) Cross section of decay 2 ft. above conks.

cultural means.—State University of New York, College of Forestry, Syracuse, N. Y.

An Improved Method of Isolating Fusarium spp. from Plant Tissue. R. H. STOVER AND B. H. WAITE. The presence of *Fusarium oxysporum* f. *cubense* in woody root and stem tissues of host carriers could be an important factor in its surviving various soil treatments designed to eradicate the fungus. Isolation of *Fusarium* spp. from such tissue has often given negative or variable results. In many cases other more rapidly growing fungi and bacteria become dominant and interfere with the determination of species present.

It was observed[1] that the Panama disease *Fusarium* from colonized dead banana root tissue developed abundantly and rapidly after the root pieces were finely cut in water in a Waring Blendor and transferred to plates of peptone-dextrose agar (pH 6.3) containing rose bengal and 60α per ml. of streptomycin[2]. Further studies were initiated to compare direct plating of tissue versus the "blending" method for the isolation of *Fusarium* spp. The amount of tissue to be used for blending and plating must be determined by trial. In most instances, pieces of stalks, roots, or petioles ½–1½ in. long depending on thickness and proximity to the roots, or 2 gm. of small roots were added to 100 ml. of water and blended 1–2 minutes. One-half or .25 ml. portions of the mixture were transferred to plates for culturing. If the root suspension was too concentrated, bacterial growth sometimes developed on the surface of the plates with certain types of blended tissue. Nevertheless, *Fusarium* spp. colonies usually developed as thin, webby growths over the bacterial layer. Streaking of a small amount of this growth on freshly poured peptone-dextrose agar plates resulted in the rapid development of *Fusarium* spp. colonies free of bacteria. Decayed pieces of banana roots from water saturated soil yielded abundant *Fusarium* spp. colonies using this method, whereas with direct plating, dense localized bacterial growth inhibited fungus development. In most cases where 1 or 2 minutes surface sterilization of sound tissue with mercuric chloride was undertaken, little difficulty with bacteria was encountered. Also, by increasing the concentration of streptomycin in the peptone-dextrose agar, bacterial growth can be reduced to a minimum without affecting fungus growth.

Previous studies[3] have indicated that several grass species can act as carriers of *F. oxysporum* f. *cubense*. When roots and crowns of these grasses were surface sterilized for 2 minutes in mercuric chloride (1:1000) and then blended they yielded numerous colonies of *Fusarium* within 3 days after plating. Similarly sterilized roots plated directly onto the agar yielded few or no colonies after 5 days. To determine the presence or survival of the fungus in those roots plated directly, pieces yielding no fungus colonies were lifted from the agar plates and stripped of their outer covering of cortical cells. The stele was then surface sterilized 2 minutes, blended for about 2 minutes, and plated at .5 ml. of suspension per plate. At 3 days after plating occasional colonies appeared,

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[2] Martin, James P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Science 69: 215-232.

[3] Waite, B. H. and V. C. Dunlap. 1953. Preliminary Host Range Studies with Fusarium oxysporum f. cubense. U.S. Dept. Agr. Pl. Dis. Rptr. 37: 79-80.

indicating survival of *Fusarium* within the stele but inability to penetrate outward through the cortex and into the agar. Whether this inability is directly due to mechanical impedence or rather a result of biological antagonisms is unknown.

Fusarium wilt of tobacco was studied using this method for purposes of comparison with the banana wilt organism. When pieces of tobacco stalks or petioles, after surface flaming or treatment with mercuric chloride, were plated directly, *F. oxysporum* f. *nicotianae* developed poorly or not at all. In many instances this species was overgrown with a *Fusarium* spp. not of the Elegans group. In contrast, plates containing portions of the blended tissue had numerous well-defined *Fusarium* spp. colonies among which the Elegans type could be easily identified. In some cases where *Fusarium* was not obtained from the lower leaf petioles by direct plating, colonies developed when the blended tissue was plated. By blending and plating various portions of wilted tobacco plants, 2–3 weeks after wilt symptoms first appeared, it was determined that *F. oxysporum* f. *nicotianae* is concentrated in the larger roots and the lower stock up to the first leaf petiole. The fungus was seldom found in the stalk 3 in. or more above the ground.

This method of isolating *Fusarium* spp. is adaptable to a study of the rate and extent of Fusarium growth in pure or mixed cultures in various plant tissues and in vascular wilt diseases of plants. The relationship of secondary or associated invaders to the primary causal agents of Fusarium wilt diseases can be studied using the above technique. Also, this method may be applicable to studies with other fungus pathogens or non-pathogens present in healthy plant tissue.—Tropical Research Department, United Fruit Company, La Lima, Honduras.

A Mechanism for the Control of Common Rootrot of Wheat.[1] S. H. F. Chinn, R. J. Ledingham, B. J. Sallans, and P. M. Simmonds. Much emphasis is placed on the role that antibiosis plays in the control of plant diseases. Essentially, the theory is that antagonistic organisms, or antibiotic substances formed by them, inhibit the development of pathogens. However, the application of this hypothesis to the control of common rootrot of wheat caused by *Helminthosporium sativum* and other fungi has given only limited success. More recent studies have indicated that the biological control of common rootrot is effected in a basically different manner from that recognized at present.

Wheat seedlings grown in crocks in the greenhouse showed a decrease of infection in artificially inoculated or naturally contaminated soil when the soil was amended with soybean meal. Normally, such control would be attributed to an increase in the activity of the microflora of the soil as a result of the organic supplement. However, by means of a newly developed slide technique, performed in conjunction with the seedling tests, it was possible to demonstrate that conidia of the causal organism, *Helminthosporium sativum*, remained in a dormant state in natural soil. On the other hand the addition of soybean meal to the soil stimulated germination of the conidia, which was followed by lysis of the germ tubes.

These observations suggested a mechanism for the control of common rootrot of wheat. In order to eliminate the conidia of *H. sativum* which usually exist in a dormant state in soil, conditions should be made favorable for the germination of the pathogen by the application of a stimulant. Then lysis of the germ tubes will follow, presumably as a result of the presence of antibiotic substances in the soil. It is reasonable to expect that a conidium dormant in the soil, even though prevented from germinating by fungistatic substances, can still be a potential source of infection. The presence of a susceptible host, such as wheat, may be all that is necessary to stimulate germination and initiate an infection. Soil sanitation through germination of the conidia and lysis of the germ tubes of the pathogen prior to planting wheat is a possible means by which common rootrot may be controlled. A full report of this work will be published soon—Laboratory of Plant Pathology, c/o University of Saskatchewan, Saskatoon, Sask.

Oospore Germination in Peronospora tabacina.[1] L. H. Person[2] and G. B. Lucas.[3] The only published account of oospore germination in *Peronospora tabacina* Adam, the causal agent of tobacco blue mold, is that by Wolf et al.[4] These workers exposed diseased leaves containing oospores to alternating cold and warm temperatures for about 6 months. At the end of this time an occasional oospore produced a germ tube when bits of the stored leaf material were macerated and kept in drops of water on microscope slides. Less than 12 oospores germinated.

This note reports a type of germination of the oospores of *P. tabacina* different from that observed by Wolf et al.

In April, 1952, dead tobacco leaves that had been affected by blue mold were collected from a tobacco plant bed. Some individual leaves were found to contain more than 1,000 oospores (Fig. 1A). Many attempts were made to germinate oospores from this material, using among others the technique of Wolf

[1] Joint contribution from the Division of Bacteriology—Contribution No. 358—and the Division of Botany and Plant Pathology—Contribution No. 1286—Science Service, Department of Agriculture, Ottawa, Canada.

[1] Contribution from the Plant Pathology Laboratories, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director as Paper No. 506 of the Journal Series.

[2] Pathologist, U. S. Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Mycology and Plant Disease Survey, Raleigh, N. C.

[3] Assistant Professor of Plant Pathology, N. C. State College, Raleigh, N. C.

[4] Wolf, F. A., Ruth A. McLean, and L. F. Dixon, 1936. Further studies on downy mildew of tobacco. Phytopathology 26: 760-777.

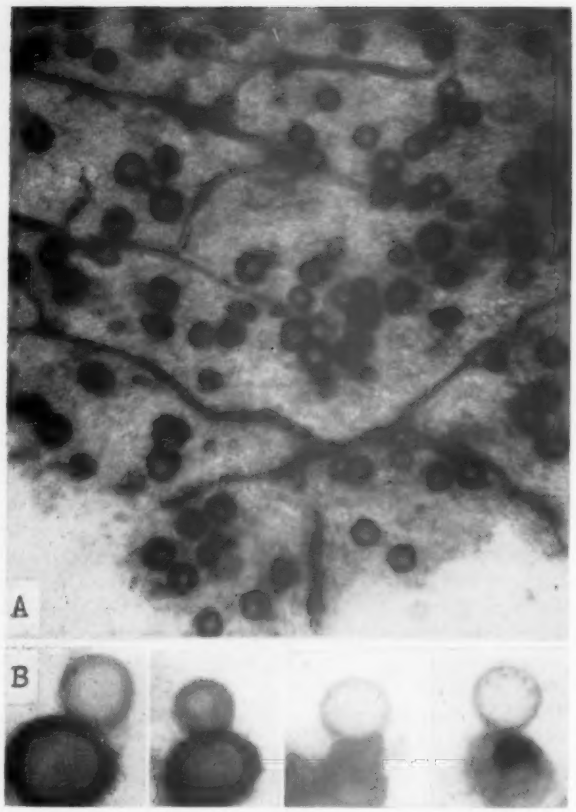

FIG. 1. Oospores of *Peronospora tabacina* Adam. A) Oospores in tobacco leaf tissue 80×. B) Germinated oospores with attached sporangia 300×. Photographs by C. J. Nusbaum.

et al. In tests involving a total of more than 100,000 oospores, there was no evidence of oospore germination. Armstrong and Sumner[5] also reported no germination of *P. tabacina* oospores even though numerous treatments and methods were tried.

In October, 1952, leaves containing oospores were placed in the top layer (⅛" deep) of sterilized soil in 4-in. clay pots. Tobacco seed were sown in the pots and transparent plastic chimneys were then placed over the pots. The seed germinated in about 1 week. Approximately 1 month after pots had been inoculated, blue mold appeared on some of the seedlings. No blue mold appeared in those pots to which no leaf material had been added. This experiment was repeated, with slight variations, 3 different times. In each case blue mold was obtained when oospores in leaf material were used as inoculum, indicating that some of the spores were viable. No previous records reporting infection under controlled conditions with oospores in leaf material have been found in the literature.

[5] Armstrong, G. M., and C. B. Sumner. 1935. Investigations on downy mildew of tobacco. South Carolina Agr. Exp. Sta. Bul. 303.

However, to determine whether or not infection originated from germinating oospores, the following experiments were performed. In April, 1953, small pieces of leaf material containing oospores were placed on the soil surface of 4-in. pots in which tobacco seedlings in the 2-leaf stage were growing. The pots were placed in a cold-room maintained at approximately 65° F. They were illuminated by daylight fluorescent light for 12 hours daily and the soil was kept moist. At intervals some of the material was examined microscopically. About 3 weeks after the oospores had been subjected to these conditions, it was noticed that some of them had formed sporangia or vesicles. In each case the rigid, thin-walled, hyaline vesicle (sporangium) was attached directly to the oospore (Fig. 1B). Sometimes 2 or rarely 3 small vesicles were attached to an oospore.

When the oospores with sporangia were placed in water, the protoplasm in the sporangium differentiated into zoospores within 20 minutes. More than 100 motile zoospores, measuring about 5μ in diameter, were formed in a single sporangium. The manner in which the zoospores escaped from the sporangium could not be definitely determined, but they were seen emerging through the wall singly and quickly.

Oospores have been germinated from material so treated 5 different times. More than 200 oospores formed sporangia. Of more than 200,000 oospores examined, none have been seen to germinate by germ tube formation.

To determine whether or not the zoospores would cause blue mold, tobacco seedlings with leaves about 1 cm. in diameter were inoculated as follows. Bits of leaf material containing germinating oospores were placed in a drop of water on a glass slide. As soon as zoospores had emerged from the sporangia and were observed swimming in the water, the slide was inverted and the drop of water touched to each of several leaves; or the drop of water containing zoospores was washed into about 10 ml. of sterile, distilled water and small drops of this suspension applied to the upper surface of several leaves by means of a pipette. The pot containing the plants was then covered with a polyethylene plastic bag and placed in a cold-room at 65° F., with alternating light and darkness every 12 hours. Conidia and conidiophores of the blue mold fungus developed in 8–10 days. Inoculations made in this manner were repeated 4 times. In all cases blue mold developed.

These observations strongly indicate that the zoospores of *P. tabacina* originating from oospores infect tobacco as part of the blue mold disease cycle. No other report has been found of oospore germination in the genus *Peronospora* by the production of a sessile zoosporangium. Studies are now underway to determine the manner by which tobacco seedlings are infected by zoospores.—North Carolina State College, Raleigh, North Carolina.

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VOLUME 43 MAY, 1953 NUMBER 5
SECTION 2

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

Directory

CONTENTS

Introduction and analysis of membership ... S. E. A. McCallan D 1
Charter members ... D 4
In Memoriam ... D 6
Past officers, editors-in-chief, and councilors ... D 7
Organization for 1953 ... D 9
Sustaining associates ... D 10
Members of the American Phytopathological Society ... D 10
Geographical index of members: United States, Territories and Possessions ... D 46
Geographical index of members: Foreign Countries ... D 53
Constitution of the American Phytopathological Society ... D 55

Subscription, $10.00 per year

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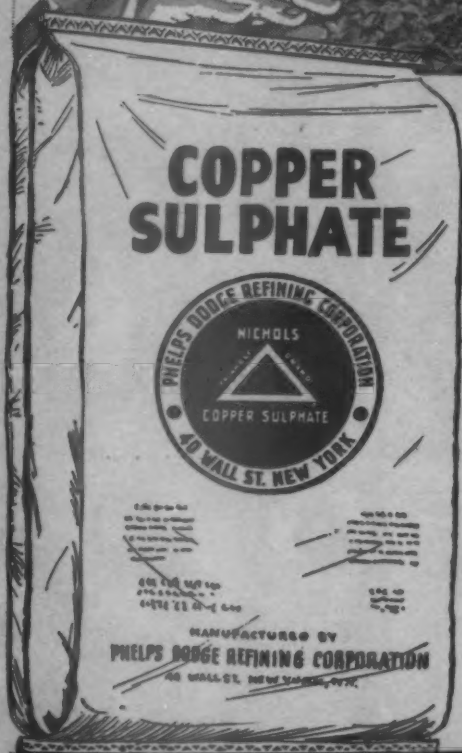

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INTRODUCTION AND ANALYSIS OF MEMBERSHIP

S. E. A. McCallan

The Directory [1] has been prepared in an attempt to provide more detailed and more usable information concerning the members of The American Phytopathological Society. It is an extension of the earlier Lists of Members. Some 1640 members and applicants for membership were asked to return a post card giving name, title, address, and fields of interest. Replies were received from 1310. From these replies and from the addresses for PHYTOPATHOLOGY for those who did not reply, the main portion of the Directory has been compiled alphabetically for the members in good standing as of March 15, 1953.[2]

A geographical index of members by states and foreign countries is also provided. There is in addition various other information concerning the members and activities of the Society. The complete list is given of the past members of the Council, that is, the officers, the Editors-in-Chief, the Councilors-at-large, and the Divisional Councilors. The In Memoriam list gives the names of deceased members since the 1948 record. The Constitution was last published as a unit in 1947; since then there have been numerous amendments. The Constitution as revised to date is accordingly presented.

From these new data on the members and from a study of the reports of Annual Meetings given in PHYTOPATHOLOGY, there is available a body of information which may be appropriately summarized in the Directory.

GROWTH OF THE SOCIETY.—The American Phytopathological Society was organized on December 30, 1908 at the Baltimore meeting of the American Association for the Advancement of Science. The first regular meeting with presentation of papers was held a year later at the Boston meeting of the A.A.A.S. On October 25, 1915, The American Phytopathological Society was incorporated under the laws of the District of Columbia. The early history of the Society has been ably and fittingly presented by the first Secretary-Treasurer, Dr. Cornelius L. Shear, in PHYTOPATHOLOGY 9: 165, 1919. The Society was founded by 130 Charter Members, whose names and original addresses we are pleased to publish herein for the first time. Twenty-eight of these Charter Members are still actively interested in the Society as Annual, Emeritus, or Life Members.

From this modest beginning the Society has had a more or less regular growth until there are now over 1600 members. This growth is shown in the accompanying graph. In addition to the fairly steady overall

rise, it will be seen that there are well defined slumps during the depression years of the early 1930's and during World War II. The latter slump was followed by a rapid rise in membership. Data are given for the time of the annual meeting in late December and include the newly elected members. Data for 1945, 1947, 1950, 1951, and 1952 are based on December 31 since the annual meetings in those years were held at markedly earlier times. The total membership for 1950, 1951 and 1952 was respectively 1533, 1623, and 1633.

CLASSES OF MEMBERSHIP.—The present classes of members are the original Charter Members, Annual, Student, and Emeritus Members, Patrons and Sustaining Associates. Beginning in 1916, "to meet the present needs of the Journal and to provide for the immediate future," the class of sustaining Life Member was created whereby the member agreed to pledge $100 in 10 consecutive annual payments. Members who contributed the $100 in one sum would become Patrons. A number of generous members participated in the plan. At present there are also 130 of these members and (Life) or (Patron) is stated after their names. However, in 1936 it was found necessary to discontinue further Life Memberships or Patrons on this basis. In 1946 a new class of Patrons was created, whose contribution should be $1,000 payable in one sum. Our 17 Sustaining Associates, or annually contributing industrial firms are listed on page D 10. The class of Emeritus Members was initiated in 1949 for members of at least 20 years' standing who would retain all rights and privileges but be exempt from dues. At present there are 14 members, mostly retired, who have availed themselves of this. The word

[1] Published as Section 2 of PHYTOPATHOLOGY Volume 43, Number 5, May, 1953.

[2] Some additional members received while in press have been added to the main alphabetical list and Geographical Index, but not to the introductory tables and text. Later additions are given only in a Supplementary List following the main listing.

(Emeritus) is given after their names. The new class of Student Membership to encourage and aid senior and graduate students with reduced dues has just been created and goes into effect this year. To date there are 174 Student Members.

DIVISIONS OF THE SOCIETY.—At present there are 5 organized divisions of the Society. In June, 1914, at Davis, California, the Western American Phytopathological Society was organized. The following year The American Phytopathological Society formally recognized geographical divisions and the Western Society became the Pacific Division. The area embraced by this oldest division is the Pacific slope west of the continental divide, including British Columbia. Meetings are held in June, usually in conjunction with the Pacific Division of the American Association for the Advancement of Science.

The Southern Division was created in 1918. The territory generally includes the states of Virginia, North Carolina, South Carolina, Tennessee, Georgia, Florida, Alabama, Mississippi, Arkansas, Louisiana, Oklahoma and Texas. Meetings are held commonly in February together with the Association of Southern Agricultural Workers.

The Canadian Division likewise was organized in 1918. It played an active part until its dissolution in 1930 upon the formation of the Canadian Phytopathological Society with the good wishes of the American Society.

The New England Division was formed in 1940. In 1946 it expanded to become the present Northeastern Division. In addition to the original 6 New England states, the states of New York, New Jersey and Pennsylvania are included within its area. Meetings are usually held in the fall or early winter.

The Potomac Division held its first annual meeting in 1944. The territory includes the District of Columbia, Maryland and Delaware and adjacent Virginia and West Virginia. Meetings are commonly held in February.

The newest division, formally organized in 1947, is the North Central, consisting of the 13 states of Ohio, Kentucky, Indiana, Michigan, Wisconsin, Illinois, Missouri, Iowa, Minnesota, North Dakota, South Dakota, Nebraska and Kansas.

Since the divisions elect their Councilors at different times and with resultant different terms of office, it was necessary to simplify their listing. Thus the Divisional Councilor is listed who held office at the time of the Annual Meeting of the year indicated.

GEOGRAPHICAL INDEX.—Institutions at one location at which there are 3 or more members are listed separately under the state. In Canada members are listed by provinces. It will be seen that there are members in all states and territories and 7 provinces of Canada. A total of 46 different foreign countries with 191 members are represented in our membership. Some members have given only a home address and it has not been possible in most of these cases to ascertain readily their institutional affiliations. With this qualification it is interesting to note that the largest centers of membership are the University of Wisconsin 64, United States Department of Agriculture, Beltsville, Maryland 59, University of Minnesota 49, Cornell University at Ithaca 42, and University of California at Berkeley 36. All branches of the University of California, i.e., Berkeley, Davis, Los Angeles and Riverside total 88. States with the largest number of members are California 171, New York 127, and Maryland 99.

TABLE 1.—*Sources of employment for members of the Society in the United States and Territories*

Classification	No. of members
State	765
Federal	180
Industrial	178
Endowed colleges and institutions	69
Federal and state cooperating	66
Retired	40
Self-employed	19

EMPLOYERS.—It is interesting to determine by whom the members of the Society are employed, that is, in its broadest aspects, the sponsors of work in plant pathology and allied fields. The sources of employment that could be determined for members in the United States and Territories are in Table 1. About 58 per cent of the members are employed under the category State. That is, they are employed by the land grant colleges, state experiment stations, and state governments. Included here are the graduate students, of which at least 174 are employed less than

TABLE 2.—*Professions or occupations of members*

Plant pathologist	910
Botanist	47
Mycologist	28
Agronomist	27
Entomologist	25
Research administrator (industrial)	25
(state, federal, endowed)	23
Plant quarantine inspector	18
Industrial manager	17
Biologist	14
Plant breeder, geneticist	14
Agricultural administrator	12
Nematologist	12
Technical sales representative	11
Horticulturist	10
Biochemist, chemist	8
Agricultural consultant	7
Bacteriologist	7
Forester, arborist	7
Superintendent field station	7
Editor	6
Microbiologist	5
Pathologist-entomologist	4
College dean, executive secretary, seed analyst, vegetable crop specialist—each	3
College president, curator, farmer, laboratory technician, plant physiologist, science teacher—each	2
Agriculturist, biophysicist, budget examiner, county agricultural agent, housewife, olericulturist, orchardist, parasitologist, soil conservationist, viticulturist each	1

TABLE 3.—*Fields of interest reported by members*

A—Diseases of crops

Vegetable (incl. tomato)	173	Tropical	17
Cereal	133	Corn	15
Forest and shade tree (incl. wood decays)	109	Roots	15
Fruit (incl. deciduous, stone, tree)	102	Sugar cane	13
Ornamentals	75	Transportation, storage, market	11
Potato	46	Bean	10
Forage (incl. grasses, pasture)	44	Sugar beet	10
Tobacco	26	Subtropical fruits	9
Small fruit (incl. strawberry, grape)	23	Soy bean	7
Citrus	22	Banana, turf—each	4
Cotton	21	Mushroom, nursery stock, peanut, rice—each	3
Field	20	Miscellaneous	20

B—Microorganisms

Mycology (incl. special groups)	91	Seed-borne pathogens	13
Fungus physiology	85	Bacteriology	10
Plant parasitic nematodes	27	Microbiology	7
Fungus genetics	21	Fungus morphology, parasitology and obligate parasites—each	4
Fungus taxonomy	19		
Soil fungi (incl. soil microorganisms)	18	Medical mycology, actinomycetes—each	3

C—Viruses

Virology	118	Stone fruit viruses	20
Virus diseases	35	Insect vectors	19

D—Other plant pathology

General plant pathology	61	Pathological plant anatomy and histology	9
Teaching	20	Gas injury, air pollution	7
Bacterial diseases	20	Extension plant pathology	6
Physiology parasitism, host parasite relations	18	Disease forecasting, biological antagonism—each	5
Plant disease survey	12	Non-parasitic diseases, pathological physiology, research methods, rust diseases, wilt diseases, foreign plant diseases—each	3
Epidemiology and ecology of disease	10		
Fungus diseases	9		

E—Control measures

Fungicides	204	Plant disease control	11
Plant breeding, disease resistance	116	Agricultural chemicals, pesticides	9
Antibiotics	44	Plant quarantines, regulating work	9
Chemotherapy (incl. systemic fungicides)	17	Fungicidal action	6
Soil treatments	16	Nematocides	6
Insecticides	13	Application machinery	4
Herbicides	12	Bacteriocides	2
Seed treatments	12		

F—Allied interests

Industrial mycology, industrial microbiology, deterioration	19	Histology, radiation biology—each	4
		Botany, mushroom culture, plant biochemistry—each	3
Plant physiology, nutrition	16	Cytology, electron microscopy, plant tissue culture—each	2
Entomology	8		
Forestry	5	Miscellaneous	42

two-thirds time, i.e., Student Members. It will be noted that as many members, 14 per cent, are employed by industry as by the federal government (exclusive of those stationed at state experiment stations). The federal positions are, of course, mostly in the United States Department of Agriculture. Industrial positions are mostly chemical and agricultural. The endowed colleges, institutions, and foundations employ only 5 per cent of the membership. Cooperative positions with the United States Department of Agriculture and the state experiment stations also account for 5 per cent. About 3 per cent of the members are retired and 1.5 per cent self-employed.

PROFESSIONS OR OCCUPATIONS.—It has been possible to identify the professions or occupations of some 1270 members from among those who returned the Directory cards. These are listed in Table 2 under 42 different headings. In some cases members have more than one profession; however, the member is listed only under the current or main profession. For ex-

ample, some plant pathologists have become administrators and are listed as such. The administrators in fields broader than plant pathology (or plant pathology and botany), insofar as possible, have been classified either as research or non-research executives. The former are listed as Research Administrators of state, federal or endowed institutions (including experiment station directors and assistant directors, heads of United States Department of Agriculture divisions, and supervisors and coordinators of endowed institutions, etc.) or as industrial research administrators (including research directors, heads, supervisors, technical directors, etc.). The non-research administrators are recorded as Executive Secretaries, Agricultural Administrators (federal and endowed programs) and Industrial Managers (including president and vice president). College president or dean is listed separately.

It will be seen that 910 or 71 per cent of the members are primarily plant pathologists, i.e., practicing plant pathologists. The graduate students in plant pathology are also included here. As might be expected, botanists, mycologists, agronomists, and entomologists constitute the largest groups of other professions. The research administrators are about evenly divided between state, federal, and endowed institutions on the one hand and industry on the other, and together they constitute a fairly large group. The Society is rather widely represented with the 42 professions or occupations in its membership. Of the total membership in the Society only 62 are women, about 4 per cent. However, 10 per cent of the founding Charter Members were women.

Fields of interest.—Most members returning the cards listed their fields of interest which ranged from 1–10 per member. A total of over 2200 items is recorded in Table 3. These interests in the great majority of cases are listed exactly as defined by the members. For the most part there has been no grouping. The main exception is in "Mycology" which includes not only "Mycology" as given by the members but also special groups of fungi. The same holds true on a lesser scale for "Bacteriology," "Entomology" and "Diseases of Ornamentals" which contain special interests. These fields of interest are definitely not mutually exclusive; for example there is mycology, fungus physiology, fungus taxonomy, etc., or fungicides, seed treatments, soil treatments, etc., or stone fruit diseases, stone fruit viruses and virus diseases.

Over 100 different fields of interest were reported. In order to simplify this somewhat in Table 3, these have been arranged into 6 main groups, namely: A—Diseases of Crops; B—Microorganisms; C—Viruses; D—Other Plant Pathology; E—Control Measures; and F—Allied Fields. About 42 per cent of the fields of interest are oriented on a crop disease basis, 22 per cent on causal agents and 21 per cent on control. The outstanding single fields of interest are: fungicides 9 per cent, vegetable diseases 8 per cent, cereal diseases 6 per cent, and plant breeding, forest and shade tree diseases, and fruit diseases, 5 per cent each. All classifications of fungicides including antibiotics, chemotherapy, soil and seed treatments, and fungicidal action total 14 per cent.

At some future date it will be possible to see how the relative order of interests change. Will there be more or less emphasis on diseases from a crop basis? Will the new fields such as antibiotics and chemotherapy have expanded appreciably? In the meantime an idea of the relative amount of work being carried out in some hundred different fields in plant pathology and allied fields may be gathered from the assembled data.

CHARTER MEMBERS

With Original Addresses

October 1909

Ames, Adelina. Cornell Univ., Ithaca, N. Y.
Arthur, J. C. Lafayette, Ind.
Bain, S. M. Knoxville, Tenn.
Ball, O. M. College Station, Texas
Ballard, W. S. Bur. Plant Ind., Washington, D. C.
Barre, H. W. Clemson College, S. C.
Barrett, J. T. Illinois Univ., Urbana, Ill.
Barrus, M. F. Cornell Univ., Ithaca, N. Y.
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Bessey, Ernst A. East Lansing, Mich.
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Field, Ethel C. Bur. Plant Ind., Washington, D. C.

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MILES, GEO. F. Bur. Plant Ind., Washington, D. C.
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MURRILL, W. A. N. Y. Bot. Garden, Bronx Park, N. Y.
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ORTON, W. A. Bur. Plant Ind., Washington, D. C.
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PATTERSON, FLORA W. Bur. Plant Ind., Washington, D. C.
PECK, CHAS H. State Museum, Albany, N. Y.
POLLOCK, J. B. Univ. Mich., Ann Arbor, Mich.
POOL, VENUS W. Univ. Nebr., Lincoln, Nebr.
RAND, F. V. Bur. Plant Ind., Washington, D. C.
READE, J. M. Univ. Georgia, Athens, Ga.
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Baines, Richard C. Associate Plant Pathologist, Department of Plant Pathology, University of California, Riverside, Calif. (Nematodes, nematocides, citrus diseases.)

Baker, Kenneth F. Professor of Plant Pathology and Plant Pathologist, University of California, 405 Hilgard Ave., Los Angeles 24, Calif. (Floricultural pathology, seed-borne pathogens.)

Baker, R. Ralph. Graduate Assistant and Student, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Sexuality and genetics of fungi, ornamental diseases.)

Bakker, Van Zinderen E. M. University of the Orange Free State, Bloemfontein, Union of South Africa.

Bald, John G. Professor of Plant Pathology, University of California, 405 Hilgard Ave., Los Angeles 24, Calif. (Diseases of bulbous ornamentals, virus.)

Baldwin, Ira L. Professor of Bacteriology and Vice President of Academic Affairs, University of Wisconsin, Madison 6, Wis. (Microbial physiology.)

Baldwin, Maynard M. Supervisor Chemical Research, Battelle Memorial Institute, 505 King Ave., Columbus 1, Ohio. (Fungicides.)

Ballantyne, Donald L., Jr. Research Assistant of Parasitology Section, Chemotherapy Department, Division of Mathieson Chemical Company, E. R. Squibbs and Sons, George Road, New Brunswick, N. J. (Parasitology.)

Ballard, William S. (Charter, Life) Retired Plant Pathologist, United States Department of Agriculture. 137 Cornell Ave., Fresno, Calif. (Physiological diseases.)

Bamberg, Rayburn H. Budget Examiner, Bureau of the Budget, Washington 25, D. C.

Bancroft, John B. Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Virology.)

Banfield, Walter M. Assistant Professor of Botany, Department of Botany, University of Massachusetts, Amherst, Mass. (Plant pathology.)

Bardin, Roy Edwards. Plant Pathologist, Monterey County Department of Agriculture, Salinas, Calif. (Plant disease survey, vegetable diseases.)

Barker, Henry D. Agriculturist in charge, Division of Cotton and other Fiber Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Cotton diseases and breeding.)

Barnes, Ervin H. Student, Department of Plant Pathology, Michigan State College, East Lansing, Mich.

Barnes, George L. Research Assistant and Graduate Student, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Potato diseases, microbiology, entomology.)

Barnes, Wm. Carroll. Superintendent, Clemson College Truck Experiment Station, Box 158, St. Andrew's Branch, Charleston, S. C. (Vegetable crops, breeding, culture, nutrition.)

Barnett, Horace L. Professor of Mycology and Mycologist, Agricultural Experiment Station, West Virginia University, Morgantown, W. Va. (Mycology, fungus physiology.)

Barrat, Joseph G. Plant Pathologist, Washington State Department of Agriculture, Irrigation Experiment Station, Prosser, Wash. (Virology.)

Barre, Henry W. (Charter, Life) Retired. Clemson, S. C. (Plant pathology, cotton diseases, cotton physiology.)

Barrett, James T. (Charter, Life) Division of Plant Pathology, University of California, Berkeley 4, Calif.

Barrus, Mortier F. (Charter, Life) Emeritus Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (General plant pathology.)

Barss, Howard P. (Life) Retired. Consultant in Plant Pathology and Experiment Station Administration, 6129 S. W. 45th Ave., Portland 19, Ore. (General plant pathology, experiment station administration.)

Bart, George J. Instructor, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio. (Forest pathology.)

Bartley, Clayton E. Main Street, West Upton, Mass.

Baskin, A. David. Plant Pathologist, Insecticide Division, P. M. A., Agricultural Research Center, Beltsville, Md. (Fungicides and herbicides.)

Bauer, Allen H. Assistant Professor of Plant Pathology Extension, 203 Botany Building, Pennsylvania State College, State College, Pa.

Baxter, Dow V. Professor of Forest Pathology and Botany, Laboratory Pathology in Forest Practice, School of Natural Resources, University of Michigan, Ann Arbor, Mich. (Forest pathology, geographic distribution and resupinate polypores.)

Baxter, John W. Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Diseases of forage legumes.)

Baxter, Luther W., Jr. Graduate Student, Department of Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Diseases of ornamentals.)

Beach, Walter S. (Life) Professor of Plant Pathology, Department of Botany, Pennsylvania State College, State College, Pa. (Vegetable, tobacco and mushroom diseases.)

Beale, Helen Purdy. (Emeritus) Retired. % Mrs. L. Bieringer, 210 Rocking Stone Ave., Larchmont, N. Y.

Beattie, R. Kent. (Life) Retired Principal Pathologist United States Department of Agriculture. Piney Branch Farm, Glen Mill Rd., Route 1, Rockville, Md. (Forest and shade tree diseases of United States and the Orient.)

Beauchamp, Julio T. Supervisory Plant Quarantine Inspector, Agricultural Research Administration, United States Department of Agriculture, Bureau of Plant Quarantines, Room 710, 408 Atlantic Ave., Boston 10, Mass.

Beckman, Carl H. Project Associate, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Forest diseases.)

Bejuki, Walter M. Plant Physiologist, Agricultural Chemical Section, Research and Development Division, Pennsylvania Salt Manufacturing Company, Whitemarsh Research Laboratories, Chestnut Hill Post Office, Philadelphia 18, Pa. (Plant response chemicals, herbicides.)

Bell, Frank H. Plant Pathologist, Estacion Experimental Agricola, Casilla 747, Cochabamba, Bolivia. (General.)

Bellingham, Roscoe C. Agent (Plant Pathologist), Fort Hays Experiment Station, Hays, Kans. (Virus diseases of cereals.)

Bennett, Carlyle W. Principal Pathologist, Division of Sugar Plant Investigations, United States Department of Agriculture, Box 31, Riverside, Calif. (Virus diseases.)

Beraha, Louis. Graduate Student, University of Illinois, Urbana, Ill. (Fruit and vegetable pathology, physiology of fungi.)

Berbee, John G. Graduate Student and Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison 6. Wis. (Forest pathology.)

Bergman, Herbert F. Senior Pathologist, Fruit and Nut Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, East Wareham, Mass. (Cranberry and blueberry diseases.)

Berkeley, Garven H. Plant Pathologist and Officer-in-Charge, Laboratory of Plant Pathology, Science Service, Canada, Agriculture, Box 87, St. Catharines, Ontario. (Virology.)

Bessey, Ernst A. (Charter) Retired Professor of Botany, Emeritus Dean of the Graduate School, Michigan State College, 213 University Dr., East Lansing, Mich. (Mycology, phylogeny.)

Bever, Wayne M. Agent (Pathologist), Bureau Plant Industry, Soils and Agricultural Engineering, and Professor of Crop Pathology, Department of Agronomy, University of Illinois, Urbana, Illinois. (Cereal diseases.)

Birchfield, Wray. Box 5427, University Station, Louisiana State University, Baton Rouge, La.

Bird, Julio. Assistant Plant Pathologist, Department of Plant Pathology, Agricultural Experiment Station of the University of Puerto Rico, Río Piedras, Puerto Rico. (Virus diseases of plants.)

Bird, Luther S. 218 N. Munnerlyn Dr., Bryan, Texas.

Bisby, Guy R. (Life) Commonwealth Mycological Institute, Kew, Surrey, England. (Mycology.)

Bishop, C. Franklin. Extension Plant Pathologist and Entomologist, West Virginia University, Morgantown, W. Va. (Vegetables, field crops and ornamentals.)

Bissonnette, Howard L. Research Assistant, Division of Plant Pathology, University of Minnesota, St. Paul, Minn.

Bitancourt, Agesilau A. Director General, Instituto Biologico, Sao Paulo, Brazil. (Citrus diseases, fungus physiology.)

Black, L. M. (Life) Professor of Plant Pathology, Department of Botany, University of Illinois, Urbana, Ill. (Virology, insect transmission, tumors, teaching.)

Blair, R. J. (Life) Springdale Farm, Ormstown, Quebec, Canada.

Blank, Lester M. Senior Pathologist, Bureau of Plant Industry, Biology Department, State College, N. Mex. (Cotton diseases.)

Blodgett, Earle C. Plant Pathologist, Washington State Department of Agriculture and Washington Agricultural Experiment Stations, Irrigation Experiment Station, Prosser, Wash. (Fruit tree diseases, virology.)

Bloom, James R. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases.)

Boasso, Celia. Vilardebo 1438, Montevideo, Uruguay.

Bobrov, Ruth Ann. Research Botanist, Los Angeles County Air Pollution Control District, 5201 Santa Fe Ave., Los Angeles 58, Calif. (Gas damage to plants, fungal taxonomy.)

Bockstahler, Harold W. Plant Pathologist, United States Department of Agriculture, P. O. Box 226, East Lansing, Mich. (Sugar beet pathology)

Boeshore, Irwin P. Plant Quarantine Inspector, United States Department of Agriculture, Bureau Entomology and Plant Quarantine, Division of Plant Quarantine, 601-A Custom House, Philadelphia 6, Pa. (Forest tree diseases, fruit and vegetable diseases.)

Boewe, G. H. Assistant Plant Pathologist, Illinois Natural History Survey, 391 Natural Resources Building, Urbana, Ill. (Plant disease survey, plant diseases.)

Bohn, G. Weston. Plant Pathologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Vegetable Crops, United States Horticultural Field Station, La Jolla, Calif. (Genetics and physiology of disease resistance and other economic characters in crop plants.)

Bonar, Lee. Professor of Botany, Department of Botany, University of California, Berkeley 4, Calif. (Fungus taxonomy, morphology.)

Bonde, Reiner. Plant Science Building, University of Maine, Orono, Maine.

Boone, Donald M. Project Associate, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Fungus genetics, inheritance of pathogenicity.)

Boosalis, Michael G. Department of Plant Pathology, University of Nebraska, Lincoln 1, Nebr.

Booth, J. A. Warren, Ariz.

Boothroyd, Carl W. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Vegetable and field crop diseases, teaching.)

Borders, Huey I. Plant Pathologist, Division Vegetable Crops and Diseases, Bureau Plant Industry, Soils, Agricultural Engineering, United States Department of Agriculture, Experiment Station, Tifton, Ga. (Diseases and related problems of vegetable transplant production.)

Borlaug, Norman E. Plant Pathologist, Rockefeller Foundation Agricultural Program in Mexico, Londres 45, Mexico, D.F., Mexico. (Breeding for disease resistance, cereal pathology, general microbiology, chemicals in the control of plant diseases.)

Bouchereau, Paul E. Pathologist, Division of Sugar Plant Investigations, Plant Industry Station, Beltsville, Md. (Sugarcane diseases.)

Bourne, Benjamin A. (Life) Vice President, Research, United States Sugar Corporation, Sugar Office Building, Clewiston, Fla. (Sugar cane diseases and breeding for resistance thereto, mineral nutrition of sugar cane.)

Bowers, James Basil. Plant Pathologist, Stauffer Chemical Company, Agricultural Research Laboratory, 1496 E. Fremont Rd., Mountain View, Calif. (Fungicides, host parasite relationships, mycology.)

Bowman, Donald H. Agronomist, Delta Branch Experiment Station, Stoneville, Miss. (Corn and small grain breeding.)

Boyce, John S. Professor of Forest Pathology, Osborn Botanical Laboratory, Yale University, New Haven 11, Conn. (Forest pathology.)

Boyce, John S., Jr. Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Federal Building, Asheville, N. C. (Tree diseases.)

Boyd, William I. Technical Service New England Grasselli Chemicals Department, E. I. du Pont de Nemours & Co., North Brookfield, Mass. (Fungicides.)

Boyle, John S. Assistant Professor of Plant Pathology, Department of Botany, The Pennsylvania State College, State College, Pa. (Fruit diseases, virology.)

Boyle, Lytton W. (Life) Associate Plant Pathologist, Department of Plant Pathology and Plant Breeding, Georgia Experiment Station, Experiment, Ga. (Peanut diseases.)

Bragonier, Wendell H. Professor and Head, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Diseases of woody plants.)

Brandes, Gordon A. Rohm and Haas, West Washington Sq., Philadelphia 5, Pa.

Brandwein, Paul F. 68-38 Yellowstone Blvd., Forest Hills, N. Y.

Brann, J. W. (Life) Baileys Harbor, Wis.

Braun, Alvin J. Associate Professor of Plant Pathology, Division of Plant Pathology, New York State Agricultural Experiment Station, Geneva, N. Y. (Small fruit diseases.)

Braun, Armin C. Ridgeview Rd., Princeton, N. J.

Braverman, Samuel W. Graduate Assistant, Botany Department, The Pennsylvania State College, United States Pasture Laboratory, State College, Pennsylvania.

Bremer, Hans. Oberregierungsrat, Institut für Gemüsebau der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Neuss II Land, Lauvenburg, Germany. (Disease of vegetable crops, chemical weed control.)

Brentzel, Wm. Edward. Chairman, Department of Plant Pathology, North Dakota Agricultural College, Fargo, N. Dak. (Cereal diseases, fungicides, antibiotics.)

Bretz, T. W. Plant Pathologist, Division Forest Pathology, United States Department of Agriculture, University of Missouri, Columbia, Mo. (Forest pathology.)

Brian, Charles E. District Manager, California Spray Chemical Corporation, 326 Fennville St., Fennville, Mich.

Bridgmon, George H. Assistant Director Wyoming Agricultural Experiment Station, University of Wyoming, Laramie, Wyo. (General.)

Brierley, Philip. Senior Pathologist, Bureau Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Diseases of ornamental plants, virology.)

Briggs, Fred N. Professor of Agronomy, Office of the Dean of Agriculture, University of California, Davis, Calif. (Disease resistance, cereal smuts and mildews.)

Brinkerhoff, Lloyd A. Associate Plant Pathologist, Botany and Plant Pathology Department, Oklahoma Agricultural and Mechanical College, and Cotton Division, United States Department of Agriculture, Stillwater, Okla. (Cotton diseases.)

Brisley, Harold R. Head of the Department of Agricultural Research, Phelps Dodge Corporation, Douglas, Ariz. (Sulphur dioxide injury, entomology, plant pathology.)

Broadfoot, William Craig, Director, Science Service Laboratories, Lethbridge, Alberta, Canada. (Cereal diseases.)

Bromfield, Kenneth R. Plant Pathologist, Camp Detrick, Frederick, Md. (General plant pathology.)

Brooks, A. Nelson. (Life) Plant Pathologist, Strawberry Laboratory, Florida Agricultural Experiment Station, P. O. Box 2386, Lakeland, Fla. (Breeding strawberries, ectoparasitic nematodes.)

Brooks, Charles. (Charter, Life) Library, Rollins College, Winter Park, Fla.

Brown, Clarke, Box 941, Caldwell, Idaho.

Brown, James Greenlief. (Life) Professor of Plant Pathology, University of Arizona and Plant Pathologist Agricultural Experiment Station, Emeritus, University of Arizona, Tucson 5, Ariz. (Bacterial plant diseases, antibiotics.)

Brown, (Mrs.) Pembroke H. 1104 West Daniel St., Champaign, Ill.

Brown, Nellie A. (Charter, Life) Retired, United States Department of Agriculture. 1851 Wyoming Ave., Washington, D. C. (Bacterial diseases of plants.)

Browning, John Artie. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Cereal diseases.)

Broyles, James W. Research Fellow in Plant Pathology, University of Minnesota, St. Paul 1, Minn. (Cereal diseases.)

Brozdowski, John J., Jr. Secretary-Treasurer of the Brozdowski Scientific Farms, Pine Island, New York. (Vegetable diseases.)

Bruehl, George W. Plant Pathologist, Department of Plant Breeding, Puerto Rico Agricultural Experiment Station, Rio Piedras, and Division of Sugar Plant Investigations, United States Department of Agriculture, Rio Piedras, Puerto Rico. (Root rots of cereals, diseases of sugarcane.)

Bruer, Howard L. 632 Mulberry St., Macon, Georgia.

Bruskin, Syril. Graduate Student, Department of Plant Pathology, College of Agriculture, Rutgers University, New Brunswick, N. J. (Mycology.)

Bryans, T. G. Head, Agriculture Department, Casa Turull, Muralla, 60, Havana, Cuba. (Fungicides, fungi in general.)

Bucha, Harry C. Biologist, E. I. du Pont de Nemours & Co., 101 W. 10th St., Wilmington, Delaware. (Fungicides, seed treatment.)

Buchholtz, Walter F. (Life) Department of Botany, Iowa State College, Ames, Iowa.

Bulger, Raymond O. Project Leader, Barberry Eradication for the Control of Stem Rust, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 301 Metropolitan Building, Minneapolis 1, Minn. (Cereal pathology.)

Burgess, Richard F. Graduate Student, Division of Plant Pathology and Botany, University of Minnesota, University Farm, St. Paul 1, Minn. (Diseases of nursery stock in cold storage, fungicides.)

Burk, Lawrence G. Geneticist, In Charge, Rubber Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Pearsall, Tex. (Physiology and genetics of the fungi.)

Burke, Douglas W. Graduate Student, Department of Plant Pathology, The State College of Washington, Pullman, Wash. (Field crop diseases, soil microbiology.)

Burke, Leo James. Plant Quarantine Inspector, Bureau of Entomology and Plant Quarantine, Room 743, 641 Washington St., New York 14, N. Y. (Ornamental diseases, disease control.)

Burke, O. D. Professor of Plant Pathology Extension, The Pennsylvania State College, State College, Pa.; 616 North Allen St., State College, Pa. (Diseases of potatoes, vegetables, ornamentals.)

Burkholder, Walter H. (Life) Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Bacterial diseases of plants.)

Burrell, Arthur B. (Life) Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Fruit tree nutrition and mineral deficiencies, spray machinery, fungicides.)

Bussart, J. Everett. Entomologist, Velsicol Corporation, 330 East Grand Ave., Chicago 11, Ill. (Insecticides, fungicides.)

Butler, Edward E. Research Fellow, Department of Plant Pathology, University of Minnesota, St. Paul, Minn.

Cairns, Eldon J. Assistant Nematologist, Division of Nematology, Plant Industry Station, Beltsville, Md. (Plant and soil nematodes, nematode morphology, physiology and taxonomy.)

Calavan, E. Clair. Assistant Plant Pathologist, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California. (Citrus diseases, fungicides, Prunus brown rot.)

Caldis, Panos D. Director of Agricultural Research, California Packing Corporation, 850 Thornton St., San Leandro, Calif. (Fruit and vegetable diseases.)

Caldwell, Ralph M. Professor of Plant Pathology and Head, Department of Botany and Plant Pathology, Purdue University, Lafayette, Ind. (Cereal pathology and breeding.)

Calvert, O. H. Associate Plant Pathologist, Department of Plant Physiology and Pathology, Texas Agricultural Experiment Station, College Station, Tex. (Diseases of vegetables and special oilseed crops, breeding for disease resistance.)

Cameron, H. Ronald. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Fruit tree virology.)

Campa-Pena, Carmen. Morelos 110 Sur, Gomez Palacio Dgo, Mexico.

Campbell, Leo. Associate Pathologist, Department of Plant Pathology, State College of Washington, Western Washington Experiment Station, Puyallup, Washington. (Vegetable diseases and mint rust.)

Campbell, Robert N. Research Assistant, Division of Plant Pathology, University of Minnesota, St. Paul 1, Minn. (Forest pathology, mycology.)

Campbell, William A. Senior Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, School of Forestry, University of Georgia, Athens, Ga. (Disease of forest and shade trees, root disease fungi and woodrotting fungi.)

Cannon, Orson S. Plant Pathologist, United States Department of Agriculture, Box 85, Utah State Agricultural College, Logan, Utah. (Vegetable diseases, disease resistance, genetics.)

Capoor, Sant P., Virus Pathologist, Plant Virus Research Laboratory, College of Agriculture, Poona 5, Bombay State, India. (Virology.)

Capellini, Raymond A. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Fungicidal action, forage crop diseases.)

Cardenas, Martin, Professor of Economic Botany, Facultad de Agronomia, Universidad Mayor de San Simon, Cochabamba, Bolivia. (Fungi in general, potato diseases.)

Caron, Omer. Provincial Botanist and Plant Pathologist, Department of Agriculture, Quebec, Canada. (Botany, plant pathology.)

Caroselli, Nestor E. Bartlett Tree Research Laboratory, Stamford, Conn.

Carpenter, John B. Senior Pathologist, United States Department of Agriculture, Cooperative Rubber Plant Field Station, Turrialba, Costa Rica. (Epidemiology, control.)

Carpenter, Theodore R. Acting Director of Research, Standard Fruit and Steamship Company, La Ceiba, Honduras. (Plant pathology.)

Carsner, Eubanks. Retired Principal Pathologist, Division of Sugar Plant Investigations, United States Department of Agriculture, 3920 Bandini Ave., Riverside, Calif.

Carter, Edward P. Pathologist in Charge, Fungicide and Herbicide Testing Laboratory, Production and Marketing Administration, United States Department of Agriculture, Agricultural Research Center, Beltsville, Md. (Fungicides.)

Carter, James Cedric. Plant Pathologist, Illinois State Natural History Survey, 385 Natural Resources Building, Urbana, Illinois. (Tree diseases, fungicides, antibiotics.)

Carter, R. D. 2223 Parker St., Berkeley 4, Calif.

Carter, Walter. Head, Entomology Department and Senior Scientist, Pineapple Research Institute of Hawaii, P. O. Box 3166, Honolulu 2, Hawaii. (Insect transmission of plant diseases, soil fumigation.)

Cash, Edith K. Associate Mycologist, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Taxonomy of Discomycetes, Alaskan fungi.)

Cash, Lillian C. Plant Pathologist, Vegetable Crops and Diseases, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Potato diseases.)

Cation, Donald. Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich.

Ceponis, Michael J. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, New York. (Viruses of stone fruits.)

Cetas, Robert C. Assistant Professor of Plant Pathology, Long Island Vegetable Research Farm of the Cornell University Agricultural Experiment Station, Riverhead, N. Y. (Vegetable diseases, fungicides.)

Chamberlain, Donald W. Plant Pathologist, United States Department of Agriculture, University of Illinois, 205 Davenport Hall, Urbana, Ill. (Soybean diseases, disease resistance, bacterial leafspots.)

Chambers, Ernest L. State Entomologist, Plant Pathology, Wisconsin Department of Agriculture, Madison, Wis. (Regulatory work, plant quarantine enforcement.)

Chandler, Webster A. Instructor in Plant Pathology, Department of Botany, The Pennsylvania State College, State College, Pa. (Fungicides.)

Chao, S. John. Research Director and Plant Pathologist, Imperial Seed Company, Clear Lake, Iowa. (Soybean and other field crop diseases, crop breeding.)

Chapman, Clarence M. East 4701 Fourth Ave., Spokane 15, Wash.

Chapman, R. Keith. Assistant Professor of Entomology, Department of Entomology, University of Wisconsin, Madison 6, Wis. (Insect transmission of plant diseases, insecticide-fungicide compatability.)

Chapman, Richard A. Associate Plant Pathologist, Kentucky Agricultural Experiment Station, Lexington, Ky. (Fungicides, nematode diseases.)

Charles, Vera K. (Charter, Life) 2039 New Hampshire Ave., N.W., Washington 9, D. C.

Chaves, Batista A. Instituto De Pesquisas, Agronomicas Dois Irmaos, Recife, Pernambuco, Brazil.

Cheo, Pen Ching. Guest Plant Pathologist, Division of Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Md. (Virus diseases.)

Cherewick, W. J. Plant Pathologist, Laboratory of Plant Pathology, P. O. Box 322, University of Manitoba, Winnipeg, Manitoba, Canada. (Cereal diseases.)

Chester, K. Starr. Supervisor of Agricultural and Biological Research, Battelle Memorial Institute, 505 King Ave., Columbus 1, Ohio. (General pathology, cereal rusts, crop losses, virus serology, chemotherapy.)

Childs, J. F. L. Pathologist, United States Department of Agriculture, Subtropical Fruit Field Station, Orlando, Fla. (Diseases of citrus and other subtropical fruit trees.)

Childs, Leroy. P. O. Box 89, Hood River, Ore.

Chilton, John E. Assistant Professor of Plant Pathology, Department of Biology, New Mexico College of Agriculture and Mechanic Arts, State College, N. Mex. (Pome and stone fruit diseases.)

Chilton, St. John P. Professor and Chairman, Department of Botany, Bacteriology and Plant Pathology, Pathologist and Head, Department of Plant Pathology, Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge 3, La. (Sugarcane diseases, genetics of fungi.)

Chitwood, B. G. Box 804, Lexington, Ky.

Chona, B. L. Systematic Mycologist, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. (Diseases of sugarcane and bananas, physiology of parasitism, taxonomy of fungi.)

Christensen, J. J. Professor of Plant Pathology, University Farm, St. Paul, Minn. (Cereal diseases and genetics of fungi.)

Christiansen, Dwight W. Graduate Student, Department of Plant Pathology, University of Nebraska, Lincoln, Nebr.

Christie, James R. Pathologist-Entomologist, Florida Agricultural Supply Company, 619 E. 7th Ave., Mount Dora, Fla. (Citrus and vegetable diseases.)

Chupp, Charles D. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Vegetable diseases, Cercosporae.)

Ciccarone, Antonio. Professore di Patologia vegetale, Facoltà di agraria, Via Valdisavoia 1, Catania, Italy. (Diseases of subtropical plants, tomato diseases, soil fumigation.)

Ciferri, Raffaele. (Life) Professor of Botany and Director of the Cryptogamic Laboratory, University of Pavia, P. O. Box 165, Pavia, Italy. (General phytopathology, taxonomy of fungi.)

Clara, Feliciano, M. (Life) Chief Plant Pathology Section Bureau of Plant Industry, Manila, Philippines. (Bacterial diseases of plants and industrial microorganisms.)

Claude, Father M. Professor of Plant Pathology, Department of Plant Pathology, Institut Agricole d'Oka, La Trappe, Quebec, Canada. (Fungicides, vegetable diseases.)

Clayton, Carlyle N. Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Fruit diseases.)

Clayton, Edward E. Principal Pathologist, Plant Industry Station, United States Department of Agriculture, Beltsville, Md. (Tobacco pathology and breeding.)

Clum, Floyd M. Graduate Assistant, Botany and Plant Pathology Department, Michigan State College, East Lansing, Mich.

Clum, Harold H. Professor, Department of Biological Science, Hunter College, 695 Park Ave., New York 21, N. Y. (Water relations in plants.)

Cochran, George W. Research Associate Professor of Plant Pathology, Department of Botany and Plant Pathology, Utah State Agricultural College, Logan, Utah. (Virology, electron microscopy.)

Cochran, L. C. Plant Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, cooperative with Citrus Experiment Station, University of California, Riverside, Calif. (Virus diseases of fruit trees.)

Cochrane, Vincent W. Associate Professor of Biology, Wesleyan University, Middletown, Conn. (Physiology of fungi and actinomycetes.)

Cocklin, Paul H. Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Coe, Donald M. Agricultural Experiment Station, Palmer, Alaska.

Cohen, Morris. Research Associate, Department of Botany, University of California, Los Angeles 24, Calif. (Pathological physiology, virology, pathogenesis.)

Cohen, Mortimer. Pathologist, Florida State Plant Board, Citrus Experiment Station, Lake Alfred, Fla. (Citrus diseases, virology.)

Cohen, Sylvan I. Technical Director, Gallowhur Chemical Corporation, 801 Second Ave., New York 17, N. Y. (Fungicides, ornamental diseases.)

Cohill, Roger W. Entomologist, Miller Chemical and Fertilizer Corporation, 2226 N. Howard St., Baltimore 18, Md. (Fungicides.)

Cohoon, D. Fred. Graduate Student, Department of Plant Pathology, College of Agriculture, Rutgers University, New Brunswick, N. J. (Fruit diseases, fungicides.)

Cole, J. R. Box 60, Albany, Ga.

Colley, Reginald H. Technical Director, Bernuth, Lembcke Company, Inc., 420 Lexington Ave., New York 17, N. Y.

Comegys, Wallace R. Assistant Plant Pathologist, Department of Plant Pathology, University of Delaware, Newark, Del. (Antibiotics and viruses.)

Conant, George H. Owner and Director, Triarch Products, Ripon, Wis. (Histological evidence of disease resistance.)

Conners, I. L. Mycologist, Department of Botany and Plant Pathology, Science Service Building, Ottawa, Ontario, Canada. (Plant disease survey, rusts, smuts.)

Conover, Robert A. Plant Pathologist, University of Florida, Sub-Tropical Experiment Station, Route 2, Box 508, Homestead, Fla. (Diseases of vegetables and subtropical plants.)

Converse, Richard H. Assistant Plant Pathologist and Agent, United States Department of Agriculture, Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Pecan diseases, fungus physiology.)

Cook, Allyn A. Assistant Pathologist, Department of Plant Pathology, South Dakota State College, Brookings, S. Dak. (Vegetable diseases.)

Cook, Harold T. Principal Pathologist, United States Department of Agriculture, Assistant Head, Division of Handling, Transportation, and Storage of Horticultural Crops, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md. (Post harvest diseases of fruits and vegetables, forecasting epiphytotics.)

Cooley, J. S. Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Md.

Coons, George H. (Life) Principal Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md. (Sugar beet pathology, breeding for disease resistance.)

Cooper, William E. Research Assistant Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Field crop diseases.)

Corbett, Merwin K. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Potato diseases, virology.)

Corcos, Alain. Graduate Student in Plant Pathology, Michigan State College, 907C Maple Lane, East Lansing, Mich. (Seed treatment.)

Corden, Malcolm E. Graduate Student, Botany and Plant Pathology Department, Oregon State College, Corvallis, Ore. (Forage diseases.)

Cormack, M. W. Plant Pathologist-in-Charge, Laboratory of Plant Pathology, Lethbridge, Alberta, Canada. (Forage crop diseases, low-temperature pathogens.)

Costa, Alvaro' S. Instituto Agronomico, Campinas Est. Sao Paulo, Brazil.

Cotter, Ralph U. (Life) Division of Plant Pathology, University Farm, St. Paul 8, Minn.

Couch, Houston B. Graduate Student, Department of Plant Pathology, University of California, Davis, Calif. (Vegetable diseases.)

Courtney, W. D. Division of Nematology, United States Department of Agriculture, Western Washington Experiment Station, Puyallup, Wash.

Cowan, E. Karl. East Coast Representative, Standard Agricultural Chemicals, Inc., Hoboken, N. J.; Interlaken, N. Y. (Fungicides, insecticides and herbicides.)

Cox, Carroll E. Department of Botany, University of Maryland, College Park, Md.

Cox, R. S. Associate Professor of Plant Pathology, Department of Plant Pathology, University of Delaware, Newark, Del. (Vegetable diseases, ornamental diseases.)

Craig, F. Waldo. Entomologist, State Department of Agriculture, Charleston, 5. W. Va. (Regulatory.)

Craigie, J. H. Retired Chief, Division of Botany and Plant Pathology, Department of Agriculture, Canada. 479 Kensington Ave., Ottawa 3, Ontario, Canada. (Cereal rusts.)

Crall, James M. Watermelon and Grape Laboratory, Box 321, Leesburg, Fla.

Cralley, E. M. Professor of Plant Pathology, Department of Plant Pathology, University of Arkansas, Fayetteville, Ark. (Cereal and legume diseases.)

Crandall, Bowen S., Senior Pathologist, Office of Foreign Agricultural Relations, United States Department of Agriculture, American Embassy, Havana, Cuba. (Root diseases, especially those caused by Phytophthoras.)

Critopoules, Patrocles D. Assistant Professor of Plant Pathology, University of Salonica, Salonica, Greece. (Cereal rusts, Phytophthora diseases, virus diseases of grape.)

Crittenden, H. William. Assistant Professor of Plant Pathology, Department of Plant Pathology, Delaware Agricultural Experiment Station, University of Delaware, Newark, Del. (Soil phytopathogens, field crop diseases.)

Crossan, Donald F. Graduate Student, Department of Plant Pathology, North Carolina State College, Raleigh, N. C. (Vegetable diseases.)

Cummings, Robert H. Graduate Student, Department of Plant Pathology, The State College of Washington, Pullman, Washington. (Fungus physiology, fungicides.)

Cummins, George B. Professor, Department of Botany and Plant Pathology, Agricultural Experiment Station, Purdue University, Lafayette, Indiana. (Taxonomy and biology of the Uredinales.)

Cunniff, Hilda Spink. Botanist, 404 South St., Key West, Fla. (Soil conservation, wild native plants of this area, insects and fungus diseases of plants in this area and their control.)

Cunningham, Howe S. Professor Emeritus, Plant Pathology, Cornell University, R. F. D. Calverton, N. Y.

Curl, Elroy A. Special Research Assistant, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, 385 Natural Resources Building, Urbana, Ill. (Tree diseases.)

Daines, Robert H. Professor of Plant Pathology, Department of Plant Pathology, Rutgers University, New Brunswick, N. J. (Disease of tree fruits, diseases of sweet potatoes, fungicides, atmospheric pollution.)

Dallimore, Clarence Eugene. Plant Pathologist, State of Idaho, Crop Pest Control and Research Commission, Branch Experiment Station, Aberdeen, Idaho. (Nematology.)

Damann, J. B. Route 1, Box 310, Strathmore, Calif. (Verticillium albo-atrum, trace element nutrition, X-ray induced mutations of plants.)

Dana, Bliss F. Pathologist, United States Department of Agriculture, Oregon State College, Corvallis, Ore. (Virus diseases of vegetables, curly top.)

Darby, John F. Plant Pathologist, University of Florida, Indian River Field Laboratory, P. O. Box 507, Fort Pierce, Fla. (Tomato and sweet corn diseases, fungicides, disease resistance, virology.)

Darker, Grant D. Mycologist, Ben Venue Laboratories, Inc., Bedford, Ohio. (Antibiotics, fungus physiology, taxonomy.)

Darley, Ellis F. Assistant Plant Pathologist, Department of Plant Pathology, University of California Citrus Experiment Station, Riverside, Calif. (Palm diseases, Armillaria root rot, air pollution injury.)

Darling, H. M. Department of Plant Pathology, Horticulture Building, University of Wisconsin, Madison 6, Wis.

Darrow, George M. Principal Horticulturist in Charge, Deciduous Fruit Investigations, Division of Fruit and Nut Crops and Diseases, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Diseases of deciduous fruits, especially of small fruits.)

Das, Gopinath, The Agricultural and Mechanical College of Texas, P. O. Box 6211, College Station, Tex.

Davidson, Richard S. Coordinator Agricultural and Biological Research, Battelle Memorial Institute, 505 King Ave., Columbus 1, Ohio. (Fungus physiology, industrial microbiology.)

Davidson, Ross W. Forest Pathologist, Division of Forest Pathology, United States Department of Agriculture, Stationed at Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colo. (Canker diseases, sapwood stains, and decays.)

Davidson, Thomas R. Associate Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, % University of Alberta, Edmonton, Alberta, Canada. (Virus diseases.)

Davies, Fred R. (Emeritus) Microbiologist, Rohm and Haas Company, Bristol, Pa. (Bacterial and fungus physiology, enzyme production.)

Davis, Benjamin H. Professor of Plant Pathology, Rutgers University, New Brunswick, N. J. (Vegetable diseases.)

Davis, David. Plant Pathologist, Crop Protection Institute, The Connecticut Agricultural Experiment Station, New Haven 4, Conn. (Chemotherapy, microbial physiology.)

Davis, Donald W. Research Entomologist, California Spray-Chemical Corporation, Sacramento, Calif. (Fungicides.)

Davis, Glen N. Associate Professor Vegetable Crops, Associate Olericulturist, Department Vegetable Crops, University of California, Davis, Calif. (Breeding for disease resistance in vegetables.)

Davis, Lily H. Principal Laboratory technician, Department of Plant Pathology, University of California, 405 Hilgard Ave., Los Angeles 24, Calif. (Plant pathogenic fungi.)

Davis, Spencer H., Jr. Associate Professor of Plant Pathology, Department of Plant Pathology, Rutgers University, New Brunswick, N. J. (Diseases of ornamental plants.)

Davis, V. H. Research and Control, Farm Bureau Cooperative Association, 245 North High St., Columbus, Ohio. (Diseases of fruit, vegetable, forage and field crops.)

Davy, Roger H., P. O. Box 181, Cadiz, Ky.

Dean, Horace S. Assistant Chief, Division of Plant Quarantine, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington 25, D. C. (Plant quarantine administration, quarantine phytopathology.

Dean, Jack L., Plant Pathologist, United States Sugar Plant Field Station, Meridian, Miss. (Sugar plant diseases.)

Dean, Leslie L. Assistant Plant Pathologist, Bean Disease Laboratory, University of Idaho, P. O. Box 1100, Twin Falls, Idaho. (Bean disease, breeding for resistance to virus diseases.)

Debbagh, K. A. Graduate Student, North Carolina State College, State College Station, Raleigh, N. Ca.

de Bruyn, Helena L. G. Retired Plant Pathologist, Laboratory for Phytopathology, Wageningen, Hinkeloordse weg 5, Wageningen, Netherlands. (Fungus diseases.)

Decker, Phares. Plant Pathologist, Florida Agricultural Experiment Station, Gainesville, Fla. (Breeding for disease resistance, vegetables, legume winter cover crops.)

Deems, R. E. Plant Pathologist, Velsicol Corporation, 330 East Grand Ave., Chicago 11, Ill. (Fungicides, fruit, cereal and vegetable diseases.)

Delp, Charles J. Division of Plant Pathology, College of Agriculture, University of California, Davis, Calif.

Demaree, Juan B. Retired Senior Plant Pathologist, Division of Fruit and Nuts, United States Department of Agriculture, 7008 Eversville Dr. Hyattsville, Md. (Berry crop diseases.)

de Ong, E. R. Consultant in Entomology and Plant Pathology, 926 Stannage Ave., Albany, Calif. (Insecticides, fungicides, herbicides.)

Desjardins, Paul R. Junior Plant Pathologist, Department of Plant Pathology, Citrus Experiment Station, University of California, Riverside, Calif. (Virology, diseases of citrus and avocado.)

Desrosiers, Russell. P. O. Box 13, Stafford, Conn.

DeVay, James E. Research Fellow, Department of Plant Pathology, University of Minnesota, St. Paul, Minn. (Biochemistry and physiology of plant pathogens.)

deZeeuw, Donald J. Assistant Professor (Research) Plant Pathology, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Vegetable diseases, seed treatment, cucurbit breeding.)

Diachun, Stephen. Plant Pathologist and Associate Plant Pathologist, Agronomy Department, University of Kentucky, Lexington, Ky. (Virology, tobacco diseases, virus diseases of forage legumes.)

Dickens, Lester E. Graduate Student, Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colo. (Diseases of ornamentals.)

Dickey, Robert S. Assistant Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Potato and cereal diseases.)

Dickinson, Sydney, School of Agriculture, Cambridge, England.

Dickson, B. T. (Life) Retired Chief, Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, 3 Normanby Crescent, Canberra, Australia.

Dickson, James G. Professor of Plant Pathology and Agent, Division of Cereal Crops and Diseases, University of Wisconsin and United States Department of Agriculture, Madison 6, Wis. (Field crop diseases, fungus physiology, nature of resistance.)

Dickson, Robert C. Assistant Entomologist, University of California, Citrus Experiment Station, Riverside, Calif. (Insect vectors of plant viruses.)

Diehl, William W., Mycologist, Division of Mycology and Disease Survey, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Adjunct Professor of Mycology, Department of Botany, The George Washington University, Washington, D. C.) (Morphology and taxonomy of Ascomycetes and Fungi imperfecti.)

Diener, Theodor O. Assistant Plant Pathologist, Irrigation Experiment Station, Prosser, Wash. (Stone fruit virus diseases.)

Diener, Urban L. Assistant Plant Pathologist, Department of Botany and Plant Pathology, Alabama Polytechnic Institute, Auburn, Ala. (Vegetable diseases, fungicides.)

Dietz, S. M. Chairman of Botany and Plant Pathology, Department of Botany and Plant Pathology, Oregon State College, Corvalis, Ore. (Disease resistance cereal pathology, tree diseases, teaching.)

Dill, Harold A. Graduate Student of Plant Pathology, Department of Plant Pathology, University of Arizona, Tucson, Ariz. (Virology, diseases of ornamentals.)

Diller, Jesse D. 40 N. Highland St., Arlington, Va.

Dimitman, Jerome E. Instructor of Plant Pathology, Department of Biological Science, California State Polytechnic College, San Dimas, Calif. (Viruses, truck crops.)

Dimock, A. W. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Diseases of ornamentals, fungicides.)

Dimond, Albert E. Chief, Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Conn. (Plant chemotherapy, fungus physiology, fungicides, toxic action.)

Dippenaar, Barend J. Professor of Plant Pathology, Agricultural Research Institute, University of Pretoria, Pretoria, Union of South Africa. (General plant pathology.)

Dobie, Norman D. Research Assistant, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore.

Dodge, Bernard O. (Emeritus) Plant Pathologist Emeritus, New York Botanical Garden, New York 58, N. Y. (Genetics and morphology of fungi, diseases of ornamental plants.)

Dodge, Carroll W. (Life) Professor, Henry Shaw School of Botany, Washington University and Mycologist, Missouri Botanical Garden, 2315 Tower Grove Ave., St. Louis 10, Mo. (Fungus morphology, human pathogens, lichens.)

Doolittle, Sears P. Prinicpal Pathologist, Division of Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States De-

partment of Agriculture, Plant Industry Station, Beltsville, Md. (Vegetable diseases, virology.)

Doran, William L. Research Professor of Botany, Department of Botany, University of Massachusetts, Amherst, Mass. (Soil-borne diseases of plants, fungicides.)

Dosdall, Louise T. Assistant Professor of Plant Pathology, Department of Plant Pathology, University of Minnesota, St. Paul Campus, St. Paul 1, Minn. (Diseases of ornamental plants, mycology.)

Drechsler, Charles. Pathologist, Division of Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Md. (Root-rotting oomycetes, nematode-destroying fungi, predacious fungi, saprophytic Entomophthoraceae.)

Drescher, R. F. Division of Plant Pathology, University Farm, St. Paul 1, Minn.

Drew, William B. Box 136, East Lansing, Mich.

Drolsom, Paul N. Agent, Tobacco Station, Oxford, N. C. (Tobacco diseases, tobacco breeding.)

Drummond, O. A. Caixa 834, B Horizonto, Minas, Brazil, South America.

DuCharme, Ernest P. Associate Plant Pathologist, University of Florida Citrus Experiment Station, Lake Alfred, Fla. (Citrus diseases.)

Duffield, P. C. The Rockefeller Foundation, Calle Londres 45, Mexico 6, D. F., Mexico.

Duffus, James E. Graduate Assistant, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Fruit virology.)

Dufrenoy, Jean, 2282 Union St., Berkeley 4, Calif.

Duggar, Benjamin M. (Charter) Consultant and Mycologist, Lederle Laboratories Division, American Cyanamid Company (Emeritus Professor of Plant Physiology and Plant Pathology, University of Wisconsin), Pearl River, N. Y. (Antibiotics, fungous physiology.)

Dulaney, Eugene L. Senior Microbiologist, Research and Development Division, Merck & Company, Inc., Rahway, N. J. (Physiology and genetics of micro-organisms, industrial microbiology.)

Dundas, Bjarne. Plant Breeder, Associated Seed Growers, Inc., Milpitas, Calif. (Bean diseases.)

Dunegan, John C. Principal Pathologist, Division of Fruit and Nut Crops and Diseases, United States Department of Agriculture, Plant Industry Station. Beltsville, Md. (Tree fruit diseases, rust of stone fruits, fungicides, antibiotics, and spray machinery.)

Dunleavy, John M. Assisant Professor of Plant Pathology, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Soybean diseases.)

Durbin, Richard D. Graduate Student, Department of Plant Pathology, University of California, Los Angeles 24, Calif. (Soil fungi, vascular pathogens, ornamentals.)

Dye, H. W. Plant Pathologist, Niagara Chemical Division, Food Machinery and Chemical Corporation, Middleport, N. Y. (Fungicides, fruit diseases, vegetable diseases.)

Dykstra, T. P. Agronomy and Horticulture Building, Louisiana State University, Baton Rouge, La.

Earhart, Robert W. Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Cereal Crops and Diseases, and Plant Pathologist, University of Florida, Agricultural Experiment Station, Gainesville, Fla. (Small grain disease, breeding for disease resistance in small grain crops.)

Eck, Richard V. Apartment 5, 4703 Ravenswood Rd., Riverdale, Md.

Eckess, Edgar. Instructor, Department of Botany, Miami University, Oxford, Ohio.

Eddins, Auther H. Plant Pathologist, Potato Investigations Laboratory, Agricultural Experiment Station, University of Florida, Hastings, Fla. (Potato and crucifer diseases, fungicides.)

Edgerton, Claude W. (Charter, Life) Professor Emeritus of Botany, Department of Botany, Louisiana State University, Baton Rouge 3, La. (Physiology and genetics of Glomerella, diseases of sugar cane.)

Edgington, Lloyd V. Graduate Student Assistant, Department of Plant Pathology, University of California, Davis, Calif.

Edson, Howard A. (Charter, Life, Emeritus) Retired. 3810 4th St., N. W., Washington 11, D. C.

Eide, Carl J. Professor of Plant Pathology, Department of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minn. (Diseases of vegetables, disease resistance in potatoes.)

Eisenstark, A. Associate Professor of Bacteriology, Department of Bacteriology, Kansas State College, Manhattan, Kans. (Virology, bacteriophage.)

El-Helaly, Abbas Fathi. Professor of Plant Pathology, Department of Plant Pathology, Alexandria University, Alexandria, Egypt. (Cereal diseases.)

Ellett, Clayton Wayne. Instructor in Botany and Plant Pathology, Department of Botany and Plant Pathology, The Ohio State University, Columbus 10, Ohio. (Teaching plant pathology.)

Elliott, Charlotte. (Life) Retired Plant Pathologist. Lanham, Md.

Elliott, Edward S. Assistant Plant Pathologist, Department of Plant Pathology, Bacteriology and Entomology, West Virginia University, Morgantown, W. Va. (Cereal and forage diseases.)

Ellis, Agnes M. Technical Editor, Division of Forest Pathology, Plant Industry Station, Beltsville, Md. (Forest pathology.)

Ellis, Don E. Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Vegetable diseases.)

Elmer, Otto H. Professor of Plant Pathology, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Vegetable diseases, virology.)

Emerson, Frank H. Technical Advisor, Agricultural Sales, Stauffer Chemical Company, 420 Lexington Ave., New York 17, N. Y. (Fungicides, pesticides, pathology.)

Emge, Robert G. Plant Pathologist, "C" Division, Camp Detrick, Frederick, Md.

Engelhard, Arthur W. Graduate Assistant, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa.

English, W. Harley. Associate Professor of Plant Pathology and Associate Plant Pathologist in the Experiment Station, Department of Plant Pathology, University of California, Davis, Calif. (Fruit diseases, pathogenic fungi.)

Epperly, Jean R. Research Assistant, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Cereal diseases.)

Epps, James M. Associate Plant Pathologist, West Tennessee Experiment Station, Jackson, Tenn. (Fungicides, cotton diseases.)

Epps, William M. Associate Plant Pathologist, South Carolina Agricultural Experiment Station, Clemson College Truck Station, P. O. Box 158, St. Andrew's Branch, Charleston, S. C. (Vegetable diseases, breeding.)

Erwin, Donald C. Junior Plant Pathologist, Citrus Experiment Station, University of California, Riverside, Calif. (Root diseases of forage and field crops.)

Evans, Arlyn W. E. I. du Pont de Nemours and Co., Inc., 3260 Jackson St., Corvalis, Ore.

Evans, Joseph A. Plant Pathologist, Grasselli Chemicals Department, E. I. du Pont de Nemours and Co., Wilmington, Del.

Evans, Stuart. 734 S. P. Dr., Phoenix, Ariz.

Exner, Beatrice Barbara. Associate Professor of Biology, Francis T. Nicholls Junior College of Louisiana State University, Thibodaux, La. (Rhizoctonias.)

Ezekiel, Walter N. Head Mycologist, Bureau of Ordnance, Navy Department, Washington 25, D. C. (Fungus and moisture-proofing of equipment, Phymatotrichum root rot, physiology of parasitism, Sclerotinia.)

Fairchild, S. J. 426 East Cook St., Santa Maria, Calif.

Farabee, Gary J. Research Assistant, Ohio State University, Columbus, Ohio.

Farrar, Luther L. Box 8008, University Station, Baton Rouge, La.

Faulkner, Lin R. Graduate Student, Department of Botany, Colorado Agricultural and Mechanical College, Fort Collins, Colo.

Faull, Joseph Horace. (Life) Emeritus Professor of Forest Pathology, Harvard University, 72 Fresh Pond Lane, Cambridge 38, Mass. (Forest and timber diseases, Pucciniastreae.)

Feder, William A. Assistant Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ornamentals Research Laboratory, Farmingdale, Long Island, N. Y. (Diseases of ornamental plants, storage diseases, plant parasitic nematodes.)

Feichtmeir, Edmund F. Shell Chemical Corporation, P. O. Box 2171, Denver, Colo. (Fungicides.)

Feldman, Albert W. Biologist, Naugatuck Chemical Division, United States Rubber Company, Bethany 15, Conn. (Plant chemotherapy, fungicides, host-pathogen physiology.)

Feldmesser, Julius. Associate Nematologist, Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, R. D. 2, Union Avenue, Hicksville, N. Y. (Nematocide screening, nematode life cycles, host-parasite relationships, ecology and experimental zoology of nematodes.)

Felix, Earl L. (Life) Associate Plant Pathologist, Agricultural Experiment Station, University of Tennessee, Knoxville 16, Tenn. (Resistance against strawberry root rots and Phytophthora fruit rots of tomato.)

Felton, M. W. Plant Pathologist, Nebraska Certified Potato Growers Association, Alliance, Nebr. (Potato diseases and production problems.)

Fenne, S. B. Professor of Plant Pathology (Extension). Virginia Polytechnic Institute, Blacksburg, Va.

Fenner, Lawrence M. (Life) Plant Pathologist, United States Department of Agriculture, Bureau of Entomology and Plant Quarantines, 209 River St., Hoboken, N. J. (Parasitic plant nematodes.)

Fergus, Charles L. Assistant Professor of Botany, Pennsylvania State College, State College, Pa. (Tree diseases, fungus physiology.)

Ferguson, John. Research Assistant in Plant Pathology, Department of Plant Pathology, University of California, Los Angeles 24, Calif. (Soil organisms, soil treatments.)

Ferris, Curtis A. Entomologist, Technical Service Division, Geigy Company, Inc., P. O. Box 1335, Fresno, Calif. (Insecticides, fungicides, virology, minor elements.)

Ferris, John M., III. Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Fezer, Karl D. Graduate Research Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Forage crop diseases.)

Fields, Walter S. (Life) Plant Pathologist, United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, Demarest, N. J.

Fink, Harry C. Assistant Research Professor, Plant Pathology Department, North Carolina State College, Raleigh, N. C. (Fruit tree diseases, fungi and virus.)

Finley, Arthur M. Assistant Plant Pathologist, Department of Plant Pathology, University of Idaho, Branch Experiment Station, Parma, Idaho. (Vegetable diseases.)

Fiore, Charles. Prairie View, Ill.

Fischer, George W. (Life) Professor of Plant Pathology and Chairman of the Department, State College of Washington, Pullman, Wash. (Taxonomy and biology of the smut fungi, smut diseases of plants.)

Fischetti, D. F. Industrias Quimicas Argentinas, Duperial Paseo Colon 285, Buenos Aires, Argentina.

Fisher, Francine E. Assistant Plant Pathologist, University of Florida, Citrus Experiment Station, Lake Alfred, Fla. (Citrus diseases and entomogenous fungi.)

Fitzpatrick, Hugh F. Plant Pathologist, Food Machinery and Chemical Corporation, Box 552, Riverside, Calif. (Post-harvest decay control.)

Fletcher, Donald G. Executive Secretary, Rust Prevention Association, 745 McKnight Building, Minneapolis 1, Minn. (Cereal rusts, aerobiology, agricultural production research.)

Flint, L. H. Botany Department, Louisiana State University, Baton Rouge, La.

Flippin, Reid S. Midwest Research Institute, 4049 Pennsylvania, Kansas City, Mo.

Flock, Robert A. Assistant Entomologist, Department of Entomology, University of California, Riverside, Calif. (Virus vectors.)

Flor, Harold H. Plant Pathologist, Division of Cereal Crops and Diseases, United States Department of Agriculture, State College Station, Fargo, N. Dak. (Flax diseases.)

Florentine, S. J. 90 West St., New York 6, N. Y.

Flynn, J. E. Coto Experiment Station, Cia Bonanera de Costa Rica, Golfito, Costa Rica.

Foeppel, William G. Graduate Student, Botany Department, Colorado Agricultural and Mechanical College, Fort Collins, Colorado. (Sugar beet and vegetable diseases, fungicides, seed treatments.)

Foley, Dean C. Graduate Student, Department of Botany, Pennsylvania State College, State College, Pa. (Cereal diseases, virology.)

Foll, S. C. 797 N. Milwaukee St., Milwaukee, Wis.

Folsom, Donald. Plant Pathologist, Plant Science Building, University of Maine, Orono, Maine. (Potato diseases.)

Fong, Richard B. 2409 16th St., Sacramento 18, Calif.

Forsberg, Junius L. Associate Plant Pathologist, Illinois State Natural History Survey, Urbana, Ill. (Diseases of floricultural crops, fungicides.)

Foster, A. Alfred. Professor of Biology, Piedmont College, Demorest, Ga. (Vegetable and flower diseases, fungicides.)

Foster, H. H. Associate Plant Pathologist, Department of Botany and Bacteriology, Clemson Agricultural College, Clemson, S. C. (Fruit pathology, primarily peaches, fungicides.)

Foster, Robert E., II. Associate Professor, University of Arizona Vegetable Research Center, University of Arizona Experiment Station, Mesa, Ariz. (Vegetable diseases, breeding disease resistant vegetables.)

Foster, Virginia. Graduate Assistant, Department of Botany and Plant Pathology, The Ohio State University, Columbus 10, Ohio. (Fungus physiology.)

Foster, William R. Plant Pathologist, Provincial Department of Agriculture, Parliament Buildings, Victoria, British Columbia, Canada. (Extension.)

Fowler, Marvin E. Bureau of Plant Industry Station, Beltsville, Md.

Fracker, Stanley B. Assistant to the Administrator, Agricultural Research Administration, United States Department of Agriculture, Washington 25, D. C. (Plant quarantines, plant disease control campaigns, national or international.)

Fraser, Willard S. Supervisor, Agricultural Research, Calumet and Hecla, Inc., P. O. Box 231, Calumet, Mich. (Fungicides.)

Frazier, Norman W. Lecturer and Assistant Entomologist, Department of Entomology, University of California, Berkeley 4, Calif. (Plant virus diseases, taxonomy of the Cicadellidae.)

Freeman, E. M. (Charter, Life) Retired. 2196 Carter Ave., St. Paul 8, Minn.

Freitag, Julius H. Associate Professor of Entomology, Department of Entomology and Parasitology, University

of California, Berkeley 4, Calif. (Virology, insect transmission of plant viruses.)

French, Alex M. Associate Plant Pathologist, California Department of Agriculture, Bureau of Plant Pathology, P. O. Box 1066, Riverside, Calif. (Fungus physiology, diseases of ornamentals and vegetables.)

French, David W. Highwood and Eastwood Aves., Dayton's Bluff, Route 2, St. Paul 6, Minn.

French, G. Talbot. State Entomologist and Plant Pathologist, Virginia Department of Agriculture, 1112 State Office Building, Richmond 19, Va. (Diseases of ornamentals, nursery stock, and vegetable garden plants.)

Fridlund, Paul R. Assistant State Plant Pathologist, Division of Plant Industry, State Department of Agriculture, Dairy and Food, 301 Coffey Hall, University Farm, St. Paul 1, Minn. (Stone fruit viruses.)

Friedman, Bernard A. Plant Pathologist, United States Department of Agriculture, Room 1022, 641 Washington St., New York 14, N. Y. (Market and bacterial diseases.)

Friedman, William. Plant Quarantine Inspector, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 641 Washington St., New York 14, N. Y. (Nematology.)

Froehlich, John P. Plant Pathologist, Insectary, Rohm and Haas Company, Bristol, Pa. (Fungicides.)

Fromme, F. D. (Life, Emeritus) Research Coordinator, Office of Experiment Stations, United States Department of Agriculture, Washington 25, D. C. (Administration and coordination of cooperative regional research.)

Frosheiser, Fred I. Graduate Student, Division of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minn.

Fulkerson, John F. Pathologist, West Virginia University, Experiment Station, Kearneysville, W. Va. (Fruit pathology.)

Fulton, Harry R. (Charter, Life) Retired Principal Pathologist, United States Department of Agriculture, 1732 Lanier Pl., Washington 9, D. C. (Fruit diseases.)

Fulton, Joseph P. Associate Professor of Plant Pathology, Department of Plant Pathology, University of Arkansas, Fayetteville, Ark. (Vegetable and strawberry diseases, virology.)

Fulton, Neil D. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Legume diseases.)

Fulton, Robert H. Instructor (Research) Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Small fruit diseases.)

Fulton, Robert W. Assistant Professor of Horticulture, Pathologist, United States Department of Agriculture, Department of Horticulture, University of Wisconsin, Madison 6, Wis. (Tobacco diseases, virology.)

Fushtey, Stephen G. Graduate Student, Department of Plant Pathology, Imperial College of Science and Technology, South Kensington, London S. W. 7, England. (Physiology of parasitism, fungicides.)

Futrell, Maurice C. Plant Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College, College Station, Tex. (Cereal diseases.)

Gainor, Charles. Associate Professor of Bacteriology, Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pa. (Bacterial variation, crown gall.)

Galleg'y, Mannon E. Assistant Professor of Plant Pathology, Department of Plant Pathology, Bacteriology, and Entomology, West Virginia University, Morgantown, W. Va. (Vegetable diseases, disease resistance.)

Garcés O, Carlos. Professor of Plant Pathology, Facultad Nacional de Agronomía, Medellín, Colombia. (Crop diseases, mycology.)

Garcia-Rada, Germán. Professor de Patologia Vegetal y Microbiologia Agricola, Escuela Nacional de Agricultura Box 456, Lima, Perú.

Gardner, Leo R. Manager Research and Development, California Spray-Chemical Corporation, Richmond, Calif.

Gardner, Max W. (Life) Professor of Plant Pathology, Plant Pathologist, University of California, Berkeley 4, Calif. (Diseases of truck crops.)

Gardner, Wayne S. Plant Pathologist, Crops Division, Technical Operations, Dugway Proving Grounds, Tooele, Utah. (Virology.)

Garren, Kenneth H. Associate Professor of Plant Pathology, Department of Botany and Plant Pathology, Alabama Polytechnic Institute, Auburn, Ala. (Diseases of woody plants, legume diseases.)

Gasiorkiewicz, Eugene C. Assistant Research Professor of Botany, University of Massachusetts, Waltham Field Station, Waltham, Mass. (Diseases of floriculture crops, virology, fungus physiology.)

Gaskill, John O. Plant Pathologist, Division of Sugar Plant Investigations, United States Department of Agriculture, Box 532, Fort Collins, Colorado. (Sugar beet diseases and breeding.)

Gauger, Wendell L. Graduate Student, Department of Plant Pathology, University of Idaho, Moscow, Idaho. (Fruit diseases.)

Gäumann, Ernst. Professor of Special Botany, Institute of Special Botany, Swiss Federal Institute of Technology, Universitätsstrasse 2, Zürich 6, Switzerland. (Fungi, plant pathology.)

Geise, Fred W. American Can Company, 104 South Michigan Ave., Chicago 3, Ill.

Gemmell, Louis G. Assistant Sales Manager, Insecticide Division, Geigy Company, Inc., 89 Barclay St., New York 8, N. Y. (Fungicides.)

Gerberg, Eugene J. Entomologist, Director, Insect Control and Research, Inc., Johnnycake Rd., Baltimore 7, Md. (Fungicides, insects in relation to plant diseases.)

Gerdemann, J. W. Assistant Professor of Crop Pathology, Department of Agronomy, University of Illinois, Urbana, Ill. (Diseases of forage legumes.)

Gerhold, Norman R. Plant Pathologist, Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colorado. (Fruit and vegetable diseases, cereal diseases, fungicides.)

Gibler, John W. Assistant Plant Pathologist, Rockefeller Foundation, Londres 45, Mexico 6, D. F., Mexico. (Wheat breeding, cereal diseases.)

Gibson, Frederick. President and Manager, Boyce Thompson Southwestern Arboretum, Superior, Ariz. (General.)

Gibson, Ian Alexander Scott. Plant Pathologist, Forest Department, Nairobi, Kenya, East Africa. (Fungus physiology, antibiotics, fungicides.)

Gibson, Kenneth E. Entomologist, United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, P. O. Box 218, Union Gap, Wash. (Insect vectors of virus diseases and their control.)

Giddings, N. J. (Charter, Life) Senior Pathologist, Division of Sugar Plant Investigations, United States Department of Agriculture, Post Office Box 31, Riverside, Calif. (Virology and general plant diseases.)

Gilbert, E. M. Emeritus Professor of Botany and Plant Pathology, Universtiy of Wisconsin, Birge Hall, Madison 6, Wis.

Gilgut, Constantine J. Professor of Extension Plant Pathology, Department of Botany, Clark Hall, University of Massachusetts, Amherst, Mass. (Fruit, vegetable, field crop and ornamental diseases, mycology, industrial mycology, fungicides.)

Gill, Denzeli L. Associate Plant Pathologist, United States Department of Agriculture, Bureau Plant Industry, Soils and Agricultural Engineering, Georgia Coastal Plain Ex-

perimental Station, Tifton, Ga. (Diseases of ornamental plants.)

Gilman, Joseph C. (Life) Professor of Botany, Department of Botany, Iowa State College, Ames, Iowa. (Parasitic fungi.)

Gilmer, Ralph A. Plant Pathologist, Western Agricultural Research Department, California Packing Corporation, Plant 167, 850 Thornton St., San Leandro, Calif. (Fungicides, plant breeding.)

Gilmer, Robert M. Assistant Professor of Plant Pathology, Division of Plant Pathology, New York Agricultural Experiment Station, Geneva, New York. (Virology, diseases of ornamentals.)

Gloyer, Walter O. Emeritus Associate Professor of Plant Pathology, Cornell University, 106 Lyceum St., Geneva, N. Y. (Variation of resistance.)

Godfrey, G. H. Texas Agricultural Experiment Substation 15, Weslaco, Tex.

Goenaga, A. Technical Assistant in Plant Pathology, Boyce Thompson Institute for Plant Research, Inc., 1086 North Broadway, Yonkers 3, N. Y. (Fungicides.)

Goheen, Austin C. Plant Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, New Brunswick, N. J. (Diseases of cranberries and blueberries.)

Goidànich, Gabriele. Professor of Plant Pathology, Director of Plant Pathological Institute of the University, Via F. Re 8 Bologna, Italy. (Fruit and fruit-trees diseases, mycology.)

Gold, Herbert. Room 107, Hilgard Hall, University of California, Berkeley, Calif.

Golden, Morgan. Assistant Nematologist, Division of Nematology, Plant Industry Station, Beltsville, Md.

González, Ada M. Castronovo de. Ingeniero Agrónomo, Dirección Nacional de Granos y Elevadores, Ministerio de Comercio Exterior, Paseo Colón 520, Buenos Aires, Argentina. (Cereal pests, insecticides, herbicides.)

Gonzalez, F. Eugene. Technical Service Representative, E. I. du Pont de Nemours Company, Sodus, N. Y. (Fungicides.)

Good, Harold M. Assistant Professor of Biology, Department of Biology, Queen's University, Kingston, Ontario, Canada. (Forest pathology, physiology of parasitism.)

Goode, Monroe J. Graduate Student, Department of Botany, Mississippi State College, State College, Miss. (Forest pathology, fruit and vegetable diesases.)

Gordon, Fred M. Biologist, Agricultural Chemicals Group, Stamford Laboratories, American Cyanamid Company, Stamford, Conn. (Applied plant pathology, nematology.)

Gordon, William L. Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, P. O. Box 322, University of Manitoba, Winnipeg, Manitoba, Canada. (Taxonomy, physiology and cytogenetics of the genus Fusarium.)

Gonzales Gorrondona, Alejandro. Norte 6 No. 123, Caracas, Venezuela, South America.

Gorenz, August M. % American Consul, Belem, Para, Brazil.

Goss, Robert W. Professor of Plant Pathology, Department of Plant Pathology, Agricultural Experiment Station, University of Nebraska, Lincoln, Nebr. (Diseases of shade trees and ornamentals.)

Goto, Shosuke C. Division Technical Operation, Dugway Proving Ground, Tooele, Utah. (Cereal diseases.)

Gottlieb, David. Associate Professor Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Ill. (Fungus physiology, antibiotics, actinomycetes.)

Gotz, M. B. 405 W. 23rd St., Apartment 12E, New York 11, N. Y.

Gould, Charles J. Plant Pathologist, Department of Plant Pathology, Western Washington Experiment Station, Puyallup, Wash. (Diseases of ornamental bulbs and rhododendrons, soil treatment.)

Graham, Joseph H. Plant Pathologist, United States Regional Pasture Research Laboratory, State College, Pa. (Forage diseases.)

Graham, K. M. Division of Botany and Plant Pathology, Science Service, Ottawa, Ontario, Canada.

Graham, Richard W. Agricultural Supervisor, Soil Conditioners Sales, Organic Division, Monsanto Chemical Company, 800 N. Twelfth St., St. Louis 1, Mo.

Graham, Shirl O. Department of Plant Pathology, Washington State College, Pullman, Wash.

Graham, Thomas W. Pathologist, United States Department of Agriculture, Tobacco, Medicinal and Special Crops, Bureau of Plant Industry, Florence, S. C. (Tobacco diseases, including nematode diseases.)

Grainger, John. Research and Advisory Officer in Plant Pathology, West of Scotland Agricultural College, Auchincruive, Ayr, Scotland. (Control of soil-borne disease, virology.)

Grant, Theodore J. Principal Pathologist, United States Department of Agriculture, Subtropical Fruit Field Station, 415 N. Parramore St., Orlando, Fla. (Citrus diseases, virology.)

Gratz, L. O. Assistant Director, Florida Agricultural Experiment Stations, Gainesville, Fla. (Research, administration.)

Gravatt, Annie R. Plant Pathologist, Horticultural Research Group, Plant Industry Station, Beltsville, Md. (Pathology of horticultural crops, forest pathology.)

Gravatt, G. Flippo. Senior Pathologist, Division of Forest Pathology, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Forest and shade tree diseases, imported diseases.)

Graves, Arthur Harmount. (Life) Consulting Pathologist, Connecticut Agricultural Experiment Station, and Curator Emeritus, Brooklyn Botanic Garden. Wallingford, Conn. (Forest pathology and forest genetics, breeding the chestnut for disease resistance.)

Graves, C. E. Dupont Company, Room 622, 111 Sutter St., San Francisco, Calif.

Graves, Clinton H., Jr. Assistant Professor of Plant Pathology, Mississippi State College, State College, Miss. (Diseases of ornamentals and small fruits.)

Greaney, Frank J. Director of Agricultural Department, Line Elevators Farm Service, Winnipeg, Manitoba, Canada. (Cereal diseases, fungicides.)

Green, Gordon J. Plant Pathologist, Dominion Laboratory of Plant Pathology. The University of Manitoba, Fort Garry, Manitoba, Canada. (Cereal diseases.)

Greenleaf, Walter H. Vegetable Breeder, Department of Horticulture, Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn, Alabama. (Disease resistance, virus, fungus, nematodes.)

Gregory, Charles T. Consultant, St. Louis Consolidated Flower Growers, 229 West Jewel Ave., Kirkwood 22, Mo. (Flower diesases.)

Gries, George A. Associate Professor, Department of Botany and Plant Pathology, Purdue University, Lafayette, Ind. (Fungus physiology, teaching.)

Griffith, R. B. National Alfalfa Dehydrating and Milling Company, Lamar, Colo.

Grogan, Raymond G. Assistant Professor of Plant Pathology, Assistant Plant Pathologist in the Experiment Station, Department of Plant Pathology, University of California, Davis, Calif. (Vegetable diseases.)

Grossbard, Erna. Research Assistant and Fulbright Fellow, Osborn Botanical Laboratory, Yale University, New Haven, Conn.

Groves, Ancell B. Plant Pathologist, Virginia Agricultural Experiment Station, Winchester Fruit Research Laboratory, Rural Route 3, Winchester, Virginia. (Deciduous tree fruit diseases, fungicides.)

Gruenhagen, Richard H. Plant Pathologist, Biochemical Research Department, The Dow Chemical Company,

Midland, Mich. (Fungicides, nematocides, antibiotics, mechanics of fungicidal action.)

Guba, Emil F. Research Professor of Botany, Waltham Field Station, University of Massachusetts, Waltham 54, Mass. (Fruit and vegetable diseases, fungicides, industrial mycology.)

Guimaraes, F. F. Estacao Exper. Hort., Domingos Petroline Rio Grande, Brazil.

Guiscafré-Arrillaga, Jaime. Chief, United States Agricultural Mission and Director, Centro Nacional de Agronomía, El Salvador, % United States Embassy, San Salvador, El Salvador. (Coffee diseases, fungicides.)

Guterman, Carl E. F. Director of Research, New York State Colleges of Agriculture and Home Economics, Director Cornell University Agricultural Experiment Station, Professor of Plant Pathology, Roberts Hall, New York State College of Agriculture, Cornell University, Ithaca, N. Y. (Diseases of ornamental plants, administration of agricultural research.)

Guthrie, James W. Assistant Plant Pathologist, University of Idaho, Branch Experiment Station, Aberdeen, Idaho. (Virology.)

Guy, H. G. Chemical Division, Koppers Company, Inc., 806 Koppers Building, Pittsburgh 18, Pa.

Haasis, Frank A. Professor of Plant Pathology, Plant Pathology Division of Biological Sciences, North Carolina State College of Agriculture, Raleigh, North Carolina. (Floral crops diseases).

Hadden, S. J. Plant Breeder, Coker's Pedigreed Seed Company, Hartsville, S. C. (Small grain breeding.)

Haenseler, C. M. Agricultural Experiment Station, New Brunswick, N. J.

Hafiz, Abdul. Assistant Professor Botany, Department of Botany, Punjab Agricultural College, Lyallpur, Pakistan. (Fungus physiology, smuts and blights.)

Hagborg, W. A. F. Plant Pathologist, Laboratory of Plant Pathology, P. O. Box 322, University of Manitoba, Winnipeg, Manitoba, Canada. (Bacterial diseases of plants.)

Hagedorn, Donald J. Assistant Professor of Agronomy and Plant Pathology, University of Wisconsin, Madison 6, Wis. (Pea diseases, legume viruses, virology.)

Haglund, Frances E. Department of Plant Pathology, University of Minnesota, St. Paul, Minn. (Stem rust epidemiology.)

Hahn, Glenn G. Pathologist, United States Department of Agriculture, Plant Industry, Soils and Agricultural Engineering, Division of Forest Pathology, 360 Prospect St., New Haven 11, Conn. (Forest and shade tree diseases.)

Halisky, Philip M. Graduate Research Assistant, Department of Plant Pathology, Washington State College, Pullman, Wash. (Cereal smuts and root rots.)

Hall, Dennis H. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, virology.)

Hall, William C. Arboriculturist, President of Arboreal Associates, Central Valley, N. Y. (Shade tree problems.)

Halpin, James E. Research Assistant Plant Pathology, Department of Plant Pathology, College of Agriculture, University of Wisconsin, Madison 6, Wis. (Diseases of forage crops.)

Hamilton, James M. Professor of Plant Pathology, Division of Plant Pathology, New ork State Agricultural Experiment Station, Cornell University, Geneva, N. Y. (Fruit diseases, fungicides, antibiotics, chemotherapy.)

Hampton, Jack E. Plant Pathologist, San Diego County Department of Agriculture, Box G, Old San Diego Station, San Diego 10, Calif. (Ornamental and truck crop diseases.)

Hampton, Richard O. Graduate Student, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Corn diseases, corn breeding.)

Hanley, Joseph F. Manager, Chemical Purchasing, Cooperative Seed and Farm Supply Service, Inc., Richmond, Va. (Fungicides, vegetable diseases.)

Hanna, William F. Chief, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. (Cereal diseases.)

Hannon, Chancellor I. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Vegetable diseases, imperfect fungi.)

Hansen, H. N. Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Variation and sex in fungi, diseases of forest and ornamental plants.)

Hansing, Earl D. Professor of Plant Pathology, and Plant Pathologist, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Cereal and forage crop diseases, seed treatment.)

Hanson, Earle W. Associate Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, and Agent, Division of Forage Crops and Diseases, United States Department of Agriculture, Madison 6, Wis. (Forage and cereal diseases.)

Hanson, R. Gordon. Southwestern Irrigation Field Station Box 1339, Brawley, Calif. (Disease resistance.)

Hardh, J. E. Senior Plant Pathologist, Agricultural Research Centre, Tikkurila, Finland.

Hardison, John R. Pathologist, United States Department of Agriculture, Department of Botany and Plant Pathology, Oregon Agricultural Experiment Station, Corvallis, Ore.

Hardy, John L. Plant Pathologist, Biochemical Research Laboratory, Dow Chemical Company, Midland, Mich. (Fungicide research.)

Hare, Woodrow W. Associate Professor of Plant Pathology, Mississippi State College, State College, Miss. (Diseases of vegetable crops.)

Harrar, J. George. Deputy Director for Agriculture, Division of Natural Sciences and Agriculture, The Rockefeller Foundation, 49 West 49th St., New York, N. Y. (International aspects of plant disease control.)

Harris, Marion R. Extension Plant Pathologist, Washington State College, Pullman, Wash. (Fungicides, extension methods for plant pathology.)

Harrison, Arthur L. Plant Pathologist, Plant Disease Laboratory, Texas Agricultural Experiment Station, Yoakum, Tex. (Vegetable and peanut diseases, fungicides, breeding for disease resistance.)

Harrison, Charles H. 2318 N. 38th St., Seattle 3, Wash. (Diseases of ornamental plants.)

Harrison, George J. (Retired) Agronomist, United States Department of Agriculture, U. S. Cotton Field Station, Route 1, Box 17, Shafter, Calif. (Cotton wilt and damping-off diseases.)

Harrison, K. A. Assistant Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, Kentville, Nova Scotia, Canada. (Vegetable and fruit diseases.)

Harrison, Martin B. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Soil fumigation, plant parasitic nematodes.)

Harry, John B. Assistant to Product Manager, Fine Chemicals Division, Carbide and Carbon Chemicals Company, 30 East 42nd St., New York 17, N. Y.

Hart, Helen. Professor of Plant Pathology, Department of Plant Pathology and Agricultural Botany, University of Minnesota, University Farm, St. Paul 1, Minn. (Rusts of cereals, disease resistance.)

Hartley, Carl. (Charter, Life) Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Beltsville, Md. (Forest tree diseases, wood deterioration.)

Hartman, John D. Professor of Vegetable Crops, Department of Vegetable Crops, Cornell University, Ithaca, N. Y. (Biological aspects of vegetable marketing.)

Harvey, Harry T. Graduate Student, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Fungus physiology, fungicides.)

Harvey, James V. Plant Pathologist, C Division, Technical Operations, Dugway Proving Ground, Tooele, Utah. (Lower fungi-saprolegniales, Pythiales, Fungi imperfecti.)

Harvey, John M. Associate Plant Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Route 3, Box 307, Fresno, Calif. (Market pathology, handling, transportation, and storage of horticultural crops.)

Haskell, Royal J. (Life) Extension Plant Pathologist, Coordinator Garden and Home Food Preservation Program, Extension Service, United States Department of Agriculture, Washington 25, D. C. (Plant disease control, extension work in plant pathology and horticulture.)

Haskett, William C. Associate Pathologist, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Cereal rusts.)

Hatfield, W. C. American Can Company, 111 Sutter St., San Francisco 4, Calif.

Havard, A. L. Green Cross Insecticides, 2875 Centre St., Montreal, Quebec, Canada.

Hawksworth, Frank G. Assistant Pathologist, Division of Forest Pathology, Box 523, Albuquerque, N. Mex. (Forest pathology.)

Hayden, E. B. Agent, Pathologist, United States Department of Agriculture, Department of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minn. (Cereal diseases.)

Haymaker, H. Henley. Professor of Plant Pathology, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Teaching.)

Hebert, Teddy T. Plant Pathology Department, North Carolina State College, Raleigh, N. C.

Heggestad, Howard E. Agronomist, United States Department of Agriculture, Tobacco Experiment Station, Greenville, Tenn. (Tobacco production, breeding and diseases.)

Heiberg, Barbara C. Mycologist-Histologist, Quartermaster Corps Food and Container Institute, Department of Army, 1849 W. Pershing Rd., Chicago 9, Ill. (Histological changes in dehydration of vegetables.)

Heimbeck, (Mrs.) Louise Solberg. Levanger, Norway. (Wilt problems, L. phase of bacteria.)

Held, Victor M. Research Assistant, United States Regional Pasture Research Laboratory, State College, Pa. (Mycology and fungus physiology.)

Hellman, Hans Petrus. President, Panogen, Incorporated, Ringwood, Ill. 865 Lake Shore Drive, Lake Geneva, Wis.

Helton, A. W. Department of Plant Pathology, University of Idaho, Moscow, Idaho.

Hemerick, Glen A. Plant Protection Officer, Plant Production Branch, Agriculture Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, Italy. (Forage crops diseases, legume seed production.)

Henderson, Lavaniel L. Research Assistant, George Washington Carver Research Foundation, Tuskegee Institute, Alabama. (Mycology, field crop diseases.)

Henderson, Robert G. (Life) Professor of Plant Pathology, Virginia Polytechnic Institute, Blacksburg, Va. (Diseases of tobacco, forage crops and tomatoes.)

Henderson, William J. Plant Pathology Department, University of California, Davis, Calif.

Hendrix, J. Walter. Department of Plant Pathology, Washington State College, Pullman, Wash.

Henry, A. W. University of Alberta, Edmonton, Alberta, Canada.

Henry, Berch W. Plant Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Forest Pathology, Harrison Experimental Forest, Saucier, Miss. (Forest tree diseases.)

Henson, Lawrence. Associate Agronomist and Associate Plant Pathologist, Department of Agronomy, Kentucky Agricultural Experiment Station, Lexington, Ky. (Forage crop diseases.)

Hepting, George H. Principal Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Asheville, N. C. (Forest tree diseases.)

Herr, Leonard J. Graduate Student, Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio. (Pathogenic soil fungi.)

Hesler, L. R. (Life) Professor of Botany and Dean, College of Liberal Arts, The University of Tennessee, Knoxville, Tenn. (Parasitic fungi and systematic mycology.)

Hesseltine, Clifford W. Mycologist, Northern Regional Research Laboratory, Peoria, Ill. (Taxonomy Mucorales and Streptomyces, industrial fermentation.)

Heuberger, John W. Professor and Head, Department of Plant Pathology, University of Delaware, Newark, Del. (Fungicides, control of vegetable and fruit diseases.)

Hewitt, Wm. B. Associate Professor of Plant Pathology, and Associate Plant Pathologist in the Experiment Station, Department of Plant Pathology, University of California, Davis, Calif. (Diseases of grapes and other fruits.)

Heyne, E. G. Agronomist, Department of Agronomy, Kansas State College, Manhattan, Kans. (Breeding disease resistant cereals.)

Hickman, D. D. 112 Horticultural Field Laboratory, University of Illinois, Urbana, Ill.

Higgins, B. B. (Life) Botanist, Georgia Experiment Station, Experiment, Ga. (Mycology, physiology of disease reactions, disease resistance.)

Higgins, Daniel J. Graduate Student, Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio. (Fungus physiology, genetics of fungi.)

Hilborn, Merle T. Associate Plant Pathologist, Department of Plant Pathology, Maine Agricultural Experiment Station, Orono, Maine. (Fungicides, fruit tree diseases, wood decay.)

Hildebrand, Alexander A. Senior Plant Pathologist, Science Service Laboratory, Harrow, Ontario, Canada. (Pathology of sugar beets and soybeans.)

Hildebrand, Earl M. 4810 Winfree St., Houston 4, Tex.

Hildebrandt, Albert C. Assistant Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Plant tissue cultures, bacterial diseases, methods in research.)

Hildreth, R. C. Graduate Assistant, Plant Pathology Department, University Farm, St. Paul 1, Minn.

Hill, John P. 1600 Elizabeth St., Redlands, Calif.

Himelick, Eugene B. Plant Pathologist, Natural History Survey, 385 Natural Resources Building, Urbana, Ill.

Hirschhorn, Elisa. Estacion Experimental, Central Castelav FCO, Argentina, South America.

Hirt, Ray R. Head, Department of Forest Botany and Pathology, New York State University, College of Forestry, Syracuse 10, New York. (Forest pathology, shade tree diseases, decay of forest products.)

Hitchner, L. S. National Agricultural Chemicals Association, Barr Building, 910 Seventeenth St., N. W., Washington 6, D. C.

Ho, William T. H. 870 Riverside Dr., Apt. 3C, New York 32, N. Y.

Hoadley, Alfred D. Assistant Plant Pathologist and Geneticist, Campbell Soup Company, Research Farm, Riverton, N. J. (Disease resistance tomatoes.)

Hobart, Oscar F., Jr. Industrial Fellow, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Plant pathology (stone fruit viruses), horticulture, mycology.)

Hobbs, Gordon A. General Delivery, Avon Park, Fla.

Hocker, Reginald O. Research Associate, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Fungus physiology, cytology, and virology.)

Hockey, J. Fred. Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, Kentville, Nova Scotia, Canada. (Fruit diseases, fungicides.)

Hoerner, Godfrey R. Agent (Plant Pathologist), United States Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Tobacco, Medicinal and Special Crops, and Professor, Oregon State College Cooperative Extension Service, Corvallis, Ore. (Hop diseases, fungicides.)

Hoffman, Paul F. Associate Plant Pathologist, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, 385 Natural Resources Building, Urbana, Ill. (Tree diseases, fungicides, fungus physiology.)

Hogan, William D. 1027 Nottingham Ave., Orlando, Fla. (Vegetable diseases, fungicides, virology.)

Holbert, J. R. Box 828, Funk Brothers Seed Company, Bloomington, Ill.

Holdeman, Quintin L. Associate Plant Pathologist, Pee Dee Experiment Station, Florence, S. C. (Tobacco diseases.)

Hollis, John P. Associate Plant Pathologist, Tropical Research Department, United Fruit Company, La Lima, Honduras, Central Ameriea. (Potato diseases, fungus physiology, sigatoka disease of banana.)

Holloman, Arthur Jr. Assistant Plant Pathologist, Air and Water Research, Stanford Research Institute, Palo Alto, Calif. (Fungicidal testing, smog control.)

Holmes, Francis O. Associate Member, The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York 21, N. Y. (Viral diseases, inheritance of disease resistance, protozoa in plants.)

Holmes, Francis W. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Forest and shade tree diseases, antibiotics, plant tissue cultures.)

Holton, Charles Stewart. Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Cereal Crops and Diseases, Agricultural Experiment Station, Pullman, Washington. (Cereal diseases.)

Holtzman, O. V. Plant Pathology Department, State College of Washington, Pullman, Wash.

Honey, Edwin E. Associate Professor of Plant Pathology Extension, Division of Agricultural Extension, Pennsylvania State College, State College, Pa. (Discomycetes, plant pathology.)

Hood, John J. 756 Washington St., West Warick, R. I.

Hooker, Arthur L. Assistant Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, Iowa State College, Iowa. (Corn diseases and breeding.)

Hooker, William J. Associate Professor, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Vegetable pathology and disease resistance.)

Hoppe, Paul E. Plant Pathologist, United States Department of Agriculture, University of Wisconsin, Madison 6, Wis. (Corn diseases.)

Hord, H. H. V. Plant Pathologist, Standard Fruit and Steamship Company, La Ceiba, Honduras. (Banana diseases.)

Horn, Norman L. Assistant Plant Pathologist, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Strawberry diseases, fungicides.)

Horner, Chester E. Research Assistant, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Fungus diseases.)

Horsey, Robert E. Vice President, Sindar Corporation, 330 W. 42nd St., New York 36, N. Y. (Fungicides, bactericides.)

Horsfall, James G. (Life) Director, The Connecticut Agricultural Experiment Station, New Haven 4, Conn. (Fungicidal action.)

Hotson, Hugh H. Deputy Chief, C Division, Dugway Proving Ground, Tooele, Utah. (Fungus physiology, pathologic tissues, obligate parasitism, chemotherapy.)

Hougas, R. W. Agent, Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, and Assistant Professor, Department of Genetics, University of Wisconsin, Madison 6, Wis. (Potato diseases.)

Hough, L. Frederic. Agriculturist-Suakoko, American Embassy, Monrovia, Liberia, % United States Department Mailing Room, Washington 25, D. C.

Houston, Byron R. Associate Professor of Plant Pathology, Department of Plant Pathology, Associate Plant Pathologist in the Experiment Station, University of California, Davis, Calif. (Field and forage crop diseases.)

Howard, Frank L. Professor and Research Professor of Plant Pathology, University of Rhode Island, Kingston, R. I. (Fungicides, chemotherapy, turf diseases.)

Hoyman, William G. Plant Pathologist, United States Department of Agriculture and North Dakota State Seed Department, Associate Plant Pathologist, North Dakota Agricultural Experiment Station, State College Station, Fargo, N. Dak. (Potato pathology and genetics.)

Hsi, David C. H. Plant Pathologist, Plains Substation, Clovis, N. Mex. (Wheat and sorghum diseases.)

Hubbell, Dean S. Senior Fellow, Mellon Institute of Industrial Research, 4400 Fifth Ave., Pittsburgh 13, Pa. (Fungicides.)

Hubert, Ernest E. Research Pathologist, School of Forestry, University of Idaho, Moscow, Idaho. (Forest pathology, radioisotope studies, wood pathology.)

Hubert, Frederic P. Inspector in Charge, Federal Plant Quarantine Service, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Isla Grande Airport, P. O. Box 3386, San Juan 15, Puerto Rico. (Plant quarantines, forest pathology.)

Hudgins, Harry R. Junior Plant Pathologist, Plant Disease Laboratory, Texas Agricultural Experiment Station, Yoakum, Texas. (Diseases of fruits and vegetables.)

Hudson, Roy. Oxnard Seed Company, P. O. Box 1306, Oxnard, Calif.

Huffington, Jesse M. 600 De Baugh Ave., Towson 4, Md.

Huffman, Marion D. Graduate Assistant, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Cereal diseases.)

Humphrey, Clarence J. (Charter) Retired, 824 S. K St., San Bernardino, Calif. (Mycology and physiology wood-destroying fungi.)

Humphrey, Harry B. (Life) Retired. 715 W. Fremont Ave., Mountain View, Calif.

Hung, Y. L. Graduate Student, 2428 Fox Ave., Madison, Wis.

Hungerford, Charles W. Professor and Head, Department of Plant Pathology, University of Idaho, Moscow, Idaho. (Bean diseases, cereal diseases.)

Hunt, N. Rex. Retired Plant Pathologist, United States Department of Agriculture, 121 Kirby St., Santa Cruz, Calif. (Protection from foreign plant diseases.)

Hunter, Herman A. (Life) 405 Magnolia Ave., Frederick, Md.

Hunter, Richard E. Assistant in Plant Pathology, Biology Department, State College, N. Mex. (Bacterial blight of cotton.)

Hurst, John W., Jr. Box 11, Boothbay Harbor, Maine.

Hutchins, Lee M. (Life) Head Pathologist in Charge, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Diseases of forests and fruit trees, virology.)

Hyre, Russell A. Plant Pathologist, United States Department of Agriculture, Mycology and Disease Survey, Department of Plant Pathology, University of Delaware, Newark, Del. (Disease forecasting, fungicides.)

Imle, Ernest Paul. Director, United States Department of Agriculture, Regional Rubber Station, Turrialba, Costa Rica.

Inman, Forrest G. Plant Quarantine Inspector, 530 Federal Building, Buffalo 3, N. Y. (Plant diseases.)

Ivanoff, Spas S. Plant Pathologist, Mississippi Agricultural Experiment Station, State College, Miss. (Disease resistance.)

Jackson, Elsa L. Research Assistant, Division of Plant Pathology and Botany, University of Minnesota, University Farm, St. Paul, Minn. (Fungus genetics.)

Jackson, Lyle W. R. Professor of Silviculture, School of Forestry, University of Georgia, Athens, Ga. (Forest pathology, plant histology, silvics and silviculture.)

Jacoway, Thomas H., Jr. Plant Pathologist, Florida Agricultural Supply Company, Post Office Box 208, Bradenton, Fla. (Fungicides.)

Jacques, J. Emile. Plant Pathologist, Montreal Botanical Gardens, 4101 Sherbrooke St. East, Montreal 36, Quebec, Canada. (Diseases of ornamentals.)

Janson, Blair F. Assistant Professor of Botany and Plant Pathology, Extension Plant Pathologist, Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio.

Jarnagin, W. M. Attapulgus Clay Company, Box 388, Philadelphia 5, Pa.

Jarvis, Karl H. Johnston, Iowa.

Jauch, Clotilde. Ingeniera Agrónoma, Facultad de Agronomía y Veterinaria de Buenos Aires, Argentina. (Plant pathology, especially diseases of ornamental plants.)

Jedlinski Henry. Plant Pathology Department, University of Nebraska, Lincoln, Nebr.

Jeffers, Walter F. Professor of Plant Pathology, Botany Department, University of Maryland, College Park, Md. (Diseases of small fruit, tree diseases, mycology, teaching of plant pathology.)

Jehle, Robert A. (Life) Retired. State Plant Pathologist, Department of Botany and Plant Pathology, University of Maryland, College Park, Md. (Potato diseases, spot anthracnoses caused by Elsinoë and Sphaceloma.)

Jenkins, Anna. Instituto Biologico, Caixa Postal 7119, Sao Paulo, Brazil.

Jenkins, Wilbert A. Plant Pathologist, Virginia Agricultural Experiment Station, Tobacco Research Station, Chatham, Va. (Breeding tobacco for disease resistance, mycology, nematology, virus diseases.)

Jensen, Dilworth D. Associate Professor of Entomology, 112 Agriculture Hall, University of California, Berkeley 4, Calif. (Insect vectors of plant viruses, virus diseases of ornamental plants, virus diseases of stone fruits.)

Jensen, Harold J. Assistant Professor of Plant Pathology, and Assistant Nematologist, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Nematodes, nematode diseases, and nematocides.)

Jensen, James H. Professor and Head of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C.

Jewell, Frederick E. Graduate Assistant, Department of Plant Pathology, West Virginia University, Morgantown, W. Va.

Johann, Helen. Retired. 1320 Spring St., Madison 5, Wis. (Fungus diseases of cereals.)

Johnson, Aaron G. (Life) Retired. Principal Pathologist, Division of Cereal Crops and Diseseas, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Cereal diseases.)

Johnson, E. M. (Life) Plant Pathologist, Kentucky Agricultural Experiment Station, Lexington, Ky. (Tobacco diseases.)

Johnson, Folke. Plant Pathologist, The State College of Washington, Western Washington Experiment Station, Puyallup, Wash. (Fruit diseases.)

Johnson, Herbert G. Plant Pathologist, Department of Agricultural Research, Green Giant Co., Le Sueur, Minn.

Johnson, Howard B. 1209 East Tyler, Harlingen, Tex.

Johnson, Howard W. Senior Pathologist, Division of Forage Crops and Diseases, Bureau Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Stoneville, Miss. (Forage crops diseases.)

Johnson, Leander F. Graduate Research Assistant, Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Soilborne diseases, microbial antagonism.)

Johnson, Nels J. Arborist (Graduate Student), Nels J. Johnson Tree Experts, 912 Pitner Ave., Evanston, Ill.

Johnson, Oscar H. Assistant Director, South Charleston Section, Technical Department, Westvaco Chemical Division, Food Machinery and Chemical Corporation, South Charleston, W. Va. (Organic chemistry, pesticides.)

Johnson, Samuel P. Graduate Student, Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College, College Station, Tex. (Fungus physiology.)

Johnson, T. C. P. O. Box 263, Lancaster, Pa.

Johnson, Thorvaldur, Principal Plant Pathologist, Laboratory of Plant Pathology, Winnipeg, Manitoba, Canada. (Cereal rusts.)

Johnston, Charles O. Plant Pathologist, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Cereal rusts.)

Jones, Edward D. Instructor of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Potato pathology.)

Jones, Fred R. (Life) Department of Plant Pathology, Horticulture Building, University of Wisconsin, Madison 6, Wis.

Jones, John P. Graduate Student, Department of Plant Pathology, Uuniversity of Nebraska, Lincoln, Nebr. (Virology, insect vectors.)

Jones, Leo E. Assistant Professor of Botany, Assistant Botanist, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Morphology, pathological anatomy, virology.)

Jones, Leon K. (Life) Inspector, Division of Plant Quarantines, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 901 Federal Office Building, Seattle 4, Wash. (Virus diseases.)

Jorstad, Ivar. Botanical Museum, Oslo, Norway.

Josephson, Leonard M. % E. A. Josephson, 2411 N. 9th St., Sheboygan, Wis.

Juantorena Dominguez, Julian M. Field Superintendent, Gomez Mena Sugar Mill, San Nicholas Province, Habana, Cuba.

Jump, John A. Associate Professor of Biology, University of Notre Dame, Notre Dame, Indiana. (Fungus physiology, biological antagonisms.)

Kahn, Robert P. Plant Pathologist, Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Md.

Kantzes, James G. Botany and Plant Pathology Department, University of Maryland, College Park, Md.

Karlovic, John K. Consulting Entomologist, Box 296, Benton Harbor, Mich. (Plant disease and insect pest control, chemical soil analysis.)

Karsten, Kenneth S. Vancide Department, R. T. Vanderbilt Company, Inc., 30 Winfield St., Norwalk, Conn. (Fungicides.)

Kazmaier, Harold E. Plant Pathologist, Battelle Memorial Institute, Columbus 1, Ohio. (Fungicides, disease resistance.)

Keener, Paul D. Assistant Professor of Plant Pathology, Department of Plant Pathology, College of Agriculture, University of Arizona, Tucson, Ariz. (Virology, bacterial diseases of alfalfa, diseases of lettuce.)

Keil, Harry L. Plant Pathologist, Biological Laboratories, Rohm and Haas Company, Bristol, Pa. (Fungicidal development.)

Keitt, George W. (Life) Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Fruit diseases, epidemiology and control, fungicides, fungus genetics and physiology, parasitism and disease resistance, virology, antibiotics, history.)

Keller, John R. Assistant Professor of Plant Pathology, Department of Botany, University of Maryland, College Park, Md. (Diseases of ornamentals, plant virology.)

Kelman, Arthur. Assistant Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Bacterial diseases.)

Kendrick, Edgar L. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Cereal and vegetable diseases.)

Kendrick, James B., Sr. (Life) Professor of Plant Pathology, Department of Plant Pathology, College of Agriculture, University of California, Davis, Calif. (Field and vegetable crop diseases.)

Kendrick, James B., Jr. Assistant Plant Pathologist, University of California Citrus Experiment Station, Riverside, Calif. (Vegetable diseases.)

KenKnight, Glenn, United States Horticulture Field Laboratory, Fort Valley, Ga.

Kennedy, William Herbert. Student, University of California, Berkeley, Calif.

Kent, George C. Professor of Plant Pathology and Head of Department, New York State College of Agriculture, Cornell University, Ithaca, N. Y. (Diseases of small grains, teaching.)

Keplinger, Joseph A. Graduate Student, Division of Plant Pathology and Botany, University Farm, St. Paul 1, Minn.

Kerling, Louise C. P. Professor of Plant Pathology, Phytopathological Laboratory, W. C. Scholten, 20 Javalaan, Baarn, The Netherlands. (Soil-borne diseases.)

Kern, Frank D. (Charter, Life) Professor Emeritus of Botany, Dean Emeritus of the Graduate School, The Pennsylvania State College, 140 W. Fairmount Ave., State College, Pa. (Uredinales.)

Kernkamp, Milton F. Associate Professor of Plant Pathology, Department of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minn. (Diseases of forage crops.)

Kevorkian, Arthur G. Assistant Head, Program Management Division, Technical Collaboration Branch, Office of Foreign Agricultural Relations, United States Department of Agriculture, Washington 25, D. C. (Tropical plant pathology.)

Kienholz, Jess R. Plant Pathologist, Fruit and Nut Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Agricultural Research Administration, Hood River, Ore. (Fruit diseases and fungicide investigations.)

Kiesling, Richard L. Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Cereal and forage diseases (smuts), and pathological histology.)

Kightlinger, Clifford V. (Life) Stockbridge Hall, Massachusetts State College, Amherst, Mass.

Kilpatrick, R. A. Pathologist, United States Department of Agriculture, Delta Branch Experiment Station, Stoneville, Mississippi. (Soybean diseases.)

Kincaid, Randall R. Plant Pathologist, North Florida Experiment Station, Quincy, Fla. (Tobacco diseases, breeding, soil treatments.)

King, Claude L. Extensionist Plant Pathology, Division of Extension, Kansas State College, Manhattan, Kans. (General plant pathology.)

King, Thomas H. Associate Professor of Plant Pathology, Department of Plant Pathology, University of Minnesota, St. Paul 1, Minn. (Fruit and vegetable diseases, virology, fungicides.)

Kingsolver, Charles H. Plant Pathologist, Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Md. (Cereal diseases.)

Kirby, Robert S. (Life) Professor of Extension Plant Pathology, Room 203, Buckhout Laboratory, Pennsylvania State College, State College, Pa. (Extension work on fruit and cereals.)

Kirkpatrick, Hugh C. Plant Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Hoticultural Crops Division, Tree Fruit Experiment Station, Wenatchee, Wash. (Virology, chemotherapy.)

Kitchen, E. M. Box 229, East Alton, Ill.

Kivilaan, Aleksander. Horticulturist and Plant Pathologist, Research Laboratory of Mount Arbor Nurseries, Shenandoah, Iowa.

Klemmer, H. W. Graduate Student, Department of Bacteriology, University of Wisconsin, Madison 6, Wis. (Antibiotics in the control of bacterial plant diseases.)

Kline, David M. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Fruit diseases.)

Klomparens, William. Department of Botany, Michigan State College, East Lansing, Mich.

Klos, Edward J. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Virology, stone fruits.)

Kneebone, Leon R. Assistant Professor of Botany, Department of Botany, The Pennsylvania State College, State College, Pa. (Mushroom culture, phytopathogenic bacteriology.)

Knorr, L. Carl. Plant Pathologist, State Plant Board of Florida, Citrus Experiment Station, Lake Alfred, Fla. (Citrus diseases, virology, relations of insects to plant diseases.)

Knote, Charles E. Manager Cape Chemical Company, Cape Girardeau, Mo. (Fungicides used in production of food.)

Koehler, Benjamin. Professor of Crop Pathology, Department of Agronomy, 210 Davenport Hall, University of Illinois, Urbana, Ill. (Diseases of cereal crops.)

Kolk, Laura A. Associate Professor Botany, Department of Biology, Brooklyn College, Brooklyn 10, N. Y. (Mycology, phycology, general botany.)

Kommedahl, Thor. Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, The Ohio State University and the Ohio Agricultural Experiment Station, Wooster, Ohio. (Cereal diseases.)

Konzak, Calvin F. Associate Geneticist, Brookhaven National Laboratory, Upton, Long Island, N. Y.

Kortsen, Robert A. University of California Extension Service, Farm Advisor, Imperial County, Court House, El Centro, Calif. (Diseases of field crops and vegetables.)

Kramer, Moyses. Instituto Biologico, Caixa Postal 2821, Sao Paulo, Brazil.

Krause, Robert L. Plant Pathologist, Agricultural Chemicals Section, Grasselli Chemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Del. (Agricultural and industrial fungicides.)

Kreitlow, Kermit W. Senior Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Plant Industry Station, Beltsville, Md. (Diseases of forage grasses and legumes.)

Kreutzer, William A. Head, Department of Plant Pathology, Shell Agricultural Laboratory, Post Office Box 1531, Modesto, Calif. (Soil fumigants, fungicides.)

Krister, Charles J. Manager, Agricultural Product Development Section, Grasselli Chemicals Department, E. I. DuPont de Nemours & Co., Wilmington, Del. (Fungicides, pesticides, market potentials.)

Kunkel, Louis O. Member Emeritus, The Rockefeller Institute for Medical Research, 66th St. and York Ave. New York 21, N. Y. (Virology.)

Kuntz, James E. Department of Plant Pathology, Horticulture Building, University of Wisconsin, Madison 6, Wis.

Kuss, Frederick R. Plant Pathologist, Technical Representative, California Spray-Chemical Corporation, 21 Winter St., Laconia, N. H.

Lachmund, Harry G. Box 422, Carmel, Calif.

Lackey, Charles F. Plant Pathologist, Division of Sugar Plant Investigations, United States Department of Agriculture, Riverside, Calif. (Virology, rust diseases.)

Laflin, Benjamin T., Jr. P. O. Box 13, Thermal, Calif.

Lair, Eugenie D. 409 Bridge St., Cynthiana, Ky.

Lal, Trilok B. Plant Pathologist, Directorate of Plant Protection, Quarantine and Storage, Plant Quarantine Station, 1082, Ganeshkhind Road, Poona 5, India. (Plant pathology in relation to quarantine, virus and nematode diseases.)

Lambert, Edmund B. Plant Pathologist, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Mushroom culture and diseases, epidemiology of cereal rusts, plant protection in Civil Defense.)

Lamey, H. A. Department of Plant Pathology, University of Wisconsin, Madison 6, Wis.

Lancaster, Francis Rhodes. Plant Pathologist, Technical Director, Davey Tree Expert Company, Kent, Ohio. (Diseases and insects of ornamental trees and shrubs.)

Lane, George H. Associate Professor, Botany and Plant Pathology, Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colo. (Potato diseases, truck crop diseases, fungicides.)

Langford, A. N. Bishop's University, Lennoxville, Quebec, Canada.

Langford, Michael H. Principal Pathologist, Division of Rubber Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, % American Embassy, San Jose, Costa Rica. (Diseases of Hevea rubber trees.)

Large, John R. Associate Plant Pathologist, Pecan Investigations Laboratory, University of Florida, Agricultural Experiment Station, Monticello, Fla. (Pecan and tung diseases, fungicides.)

Larson, Russel H. Plant Pathologist, Department of Plant Pathology, University of Wisconsin, and United States Department of Agriculture, Madison 6, Wis. (Vegetable diseases, cabbage, onions and potatoes, virology, disease resistance.)

Latham, Dennis H. Associate Pathologist, Highland Rim Experiment Station, University of Tennessee, Springfield, Tenn. (Tobacco diseases.)

Latterell, Frances Meehan, Plant Pathologist, C Division, Camp Detrick, Frederick, Md. (Cereal diseases.)

Lautz, William H. Assistant Plant Pathologist, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, United States Department of Agriculture, Beltsville, Md. (Diseases of Tobacco.)

Lawyer, Lewis O. Assistant Director Agricultural Research, California Packing Corporation, 850 Thornton St., San Leandro, Calif.

Leach, Julian G. Professor of Plant Pathology, Department of Plant Pathology, Bacteriology and Entomology, West Virginia University, Morgantown, W. Va. (Plant pathology, bacteriology, and insect transmission of plant diseases.)

Leach, Lysle D. (Life) Professor of Plant Pathology and Plant Pathologist, University of California, Davis, Calif. (Seedling diseases and fungicides.)

Lear, Bert. Nematologist, Department of Biology, New Mexico College of Agriculture and Mechanical Arts, State College, New Mexico. (Soil fumigation, soil organisms, diseases of vegetable crops.)

LeBeau, Francis J. Agriculturist (Plant Pathologist) United States Department of Agriculture, Office of Foreign Agricultural Relations. Instituto Agropecuario Nacional, Guatemala, Guatemala. (General.)

Lebeau, J. B. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis.

Leben, Curt. Assistant Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Antibiotics, fungicides, microbial genetics, physiology of fungi.)

LeClerg, E. L. Research Coordinator, Agricultural Research Administration, United States Department of Agriculture, Washington 25, D. C. (General pathology.)

Lee, H. Atherton. (Life) Director, Agricultural Experiment Station, Mayaguez, Puerto Rico.

Lee, Bertrand W. Assistant Plant Pathologist, Bureau of Plant Pathology, California Department of Agriculture, Sacramento 14, Calif. (General survey.)

Lefebvre, C. L. Plant Pathologist, Office of Experiment Stations, United States Department of Agriculture, Washington 25, D. C. (Diseases of forage crops and administration.)

Lehman, Samuel G. Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Cotton and soybean diseases.)

Lepore, A. 111 Marlow St., Cranston, R. I.

Leukel, Robert W. Plant Pathologist, Division of Cereal Crops and Diseases, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Cereal diseases, fungicides for their control.)

Levine, Moses N. (Life) Pathologist, United States Department of Agriculture, also Guest Professor Hebrew University, Jerusalem, Israel, Institute of Agriculture, University of Minnesota, St. Paul 1, Minn. (Cereal rusts and smuts.)

Lewis, Gwynne D. Graduate Research Fellow, Department of Botany and Plant Pathology, Purdue University, Lafayette, Ind. (Fruit diseases, fungicides.)

Lewis, Fred H. Professor of Plant Pathology, The Pennsylvania State College Fruit Research Laboratory, Arendtsville, Pa. (Fruit diseases, fungicides.)

Lewis, Ralph W. Professor of Biology, Department of Natural Science, Michigan State College, East Lansing, Mich. (Fungus nutrition and physiology, ergot, host-parasite relations.)

Lewis, Richard M. Research Associate, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Cereal diseases, fungicides.)

Leyendecker, Philip J., Jr. Associate Professor of Plant Pathology, Department of Biology, New Mexico College of Agriculture and Mechanic Arts, State College, N. Mex. (Cotton diseases, Verticillium.)

Libby, Robert R. Graduate Student Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C.

Limber, Donald P. Plant Quarantine Inspector, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 209 River St., Hoboken, N. J. (Plant pathology and mycology, virology.)

Liming, O. Neal. Plant Quarantine Officer, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D. C. Temporary address: Box 576 Agana, Guam. (Quarantine, fungicides.)

Lindberg, G. Don. Graduate Student of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable pathology, virology.)

Lindgren, Ralph M. Principal Pathologist, United States Division of Forest Pathology, Forest Products Laboratory, Madison, Wis. (Forest products and tree pathology.)

Lindner, Robert C. Plant Pathologist, Tree Fruit Experiment Station, Wenatchee, Wash. (Virology, plant physiology.)

Linford, M. B. (Life) Department of Horticulture, University of Illinois, Urbana, Ill.

Ling, Lee. Plant Pathologist, Food and Agriculture Organization of the United Nations, Viale Terme di Caracalla, Rome, Italy. (Plant pathology, mycology.)

Link, George K. K. Botany Building, University of Chicago, Chicago, 37, Ill.

Linn, Manson B. Professor of Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Ill. (Vegetable and canning crop diseases.)

Littauer, F. Simon. 17 Ruppin St., Rehovot, Israel.

Littooy, E. H. Colloidal Products Corporation, 2598 Taylor St., San Francisco 11, Calif. (Fungicides.)

Litzenberger, Samuel C. Head of the Agronomy Department, Servicio Tecnico Agricola de Nicaragua and Research Advisor in Agriculture, Institute of Inter-American Affairs, Managua, Nicaragua. (Agronomic crop diseases, fungicides.)

Livingston, A. M. Chemical Insecticide Corporation, 57 13th St., Brooklyn 15, N. Y.

Livingston, Jesse E. Professor of Plant Pathology and Plant Pathologist, University of Nebraska, Lincoln 3, Nebr. (Diseases of cereals, systemic fungicides.)

Locke, Seth Barton. Associate Professor of Plant Pathology and Associate Plant Pathologist, Department of Plant Pathology, State College of Washington, Pullman, Washington. (Potato diseases, chemotherapy of virus diseases, crown gall development.)

Locke, Warren F. Waller-Franklin Seed Company, Guadalupe, Calif.

Lockwood, John L. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Antibiotics, fungicides.)

Loegering, William Q. Plant Pathologist, United States Department of Agriculture, Interamerican Institute of Agricultural Sciences, Turrialba, Costa Rica. (Diseases of abaca.)

Logsdon, Charles E. Graduate Student, Division of Plant Pathology, University Farm, St. Paul 1, Minn. (Potato diseases, virology.)

Loh, T. C. (Life) Division of Agronomy, Agricultural Experiment Station, Shatong, Liuchow, Kwangsi, China.

Lohman, Marion L. Associate Professor of Botany, University of Hawaii, Honolulu 14, Hawaii. (Teaching, research methods.)

Lombard, Mrs. Frances F. Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Cultural studies of wood-rotting fungi.)

Long, William G. Washington Cottage, Braddock Heights, Md.

Lorenz, Rolland C. Director of the Instituto Agropecuario Nacional, La Aurora, Guatemala City, Guatemala. (Tropical and subtropical plant diseases.)

Lownsbery, Benjamin F. Assistant Plant Pathologist, The Connecticut Agricultural Experiment Station, New Haven 4, Conn. (Nematology, plant pathology.)

Lowther, Conley V. Plant Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Cereal Crops and Diseases, Beltsville, Md. (Cereal diseases.)

Lucas, Eugene H. Professor of Horticulture, Department of Horticulture, Michigan State College, East Lansing, Mich. (Drug plants, basidiomycetes, antibiotics.)

Lucas, George Blanchard. Assistant Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Tobacco diseases, genetics of fungi.)

Luke, Herbert H. Graduate Student, Department of Botany and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Antibiotics.)

Lutes, Dallas D. Graduate Student, Department of Botany, University of Missouri, Columbia, Mo. (Virology, bacteriology.)

Luttrell, E. S. Plant Pathologist, Department of Plant Pathology, Georgia Experiment Station, Experiment, Ga. (Diseases of forage crops and cereals, morphology of Ascomycetes.)

Lyerly, Paul J. Superintendent, Texas Agricultural Experiment Station, Substation 17, Ysleta, Tex. (Cotton.)

Lyle, Eldon W. Plant Pathologist, Texas Rose Research Foundation, Inc., Route 9, Box 159, Tyler, Tex. (Rose diseases, rose culture.)

Lyle, James A. Assistant Plant Pathologist, Department of Botany and Plant Pathology, Alabama Polytechnic Institute, Auburn, Ala. (Legume and cereal diseases, fungus physiology.)

Macdonald, Kenneth H. Graduate Student Assistant, Department of Plant Pathology, New York State College of Agriculture, Cornell University, Ithaca, N. Y. (Golden nematode disease of potatoes, microbial antagonism in soil.)

Machacek, John E. Senior Plant Pathologist, Laboratory of Plant Pathology, P. O. Box 322, University of Manitoba, Winnipeg, Manitoba, Canada. (Seed-borne diseases, root diseases of cereals.)

Machado, Adelmo A. Professor of Plant Pathology and Head of the Department of Biology (Entomology, botany and plant pathology) in the Escola de Agronomia do Nordeste, Areia, State of Paraíba, Brazil. (Fungus and physiogenic diseases of plants.)

Machmer, John H. Associate Nematologist, Division Nematology, United States Department of Agriculture, Bureau Plant Industry, Georgia Coastal Plain Experiment Station, Tifton, Ga.

MacMillan, Howard G. (Life) 1725 Fifth Ave., Los Angeles 19, Calif. (Vegetable diseases, diseases of tropical plants.)

MacNair, James D. Associate Editor Agricultural Chemicals, 175 Fifth Ave., New York 10, N. Y.

MacNeill, Blair H. Associate Professor of Botany, Department of Botany, Ontario Agricultural College, Guelph, Canada. (Vegetable pathology, virology.)

Mader, Erich O. 1840 Berkeley Ave., St. Paul 5, Minn. (Plant physiology, fungus physiology.)

Magie, Robert O. Plant Pathologist, Gulf Coast Experiment Station, Bradenton, Fla. (Gladiolus disease control, soil fungicides, systemic fungicides.)

Mahoney, Charles H. 1739 H St., N. W., Washington, D. C.

Mai, William F. Professor of Plant Pathology, Cornell University, Ithaca, N. Y. (Potato diseases.)

Mains, Edwin B. (Life) Professor of Botany, Director of the Herbarium, Museums Building, University of Michigan, Ann Arbor, Michigan. (Uredinales, disease resistance, entomogenous fungi.)

Makila, Armas A. Graduate Student, Division of Plant Pathology, University Farm, St. Paul, Minn.

Malaguti, Gino. Assistant Plant Pathologist, Division de Fitopatologia, Instituto Nacional de Agricultura, Maracay, Venezuela. (Tropical crops diseases.)

Manns, Thomas F. (Charter, Life) Emeritus Professor of Plant Pathology and Soil Bacteriology, University of Delaware, Newark, Del. (Fungus and virus diseases, soil microbiology, role of copper in soils.)

Maramorosch, Karl. Assistant, The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York 21, N. Y. (Viruses and virus diseases.)

Marcano, Luis. Division de Fitotecnia, Apartado 660, Maracay, Venezuela.

Marcus, Carlton P., Jr. Plant Pathologist, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Small fruit diseases.)

Markley, Brian Bruce. Graduate Student, Department of Plant Pathology, University of California, Los Angeles, 405 Hilgard Ave., Los Angeles 24, Calif.

Marlatt, Robert B. Assistant Plant Pathologist, Department of Plant Pathology, University of Arizona, P. O. Box 751, Phoenix, Ariz. (Vegetable diseases, non-parasitic diseases.)

Marsden, David H. Assistant Professor, Research, Shade Tree Laboratories, University of Massachusetts, Amherst, Mass. (Forest, shade and ornamental tree pathology.)

Marshall, Barton H., Jr. Plant Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Diseases of ornamental plants.)

Marshall, Norton L. Graduate Student, Department of Botany, University of Maryland, College Park, Md. (Fungicides, fungus physiology.)

Marshall, Rush P. Director, Bartlett Tree Research Laboratories, 60 Canal St., Stamford, Conn. (Diseases of shade trees.)

Martin, G. Hamilton, Jr., (Life) State Rd., South Sudbury, Mass.

Martin, Helen. Junior Chemist, Rohm and Haas Company, Bristol, Pa. (Fungicides.)

Martin, William H. (Life) Agricultural Experiment Station, New Brunswick, N. J.

Martin, W. J. Associate Plant Pathologist, Louisiana State University, Baton Rouge 3, La. (Diseases of vegetable crops.)

Mason, Curtis L. Plant Pathologist, Niagara Chemical Division, Food Machinery and Chemical Corporation, Middleport, N. Y. (Fungicides, fruit diseases, cotton diseases.)

Massey, Louis M. (Life) Professor of Plant Pathology, New York State College of Agriculture, Cornell University, Ithaca, N. Y. (Diseases of ornamentals, fungicides.)

Mastenbroek, C. Phytopathologist, Plant Breeding Station, Centraal Bureau, Hoofdweg 741, Hoofddorp, Netherlands. (Plant breeding, breeding for resistance to diseases, especially of the potato.)

Matsumoto, Takashi. Emeritus Professor of Plant Pathology, Phytopathological Laboratory, National Taiwan University, Taipei, Taiwan, China. (Virology, tropical phytopathology.)

Mattei, John A. Student, Division of Plant Pathology, University of California, Berkeley, Calif.

Matz, Julius. Topsfield, Mass.

Maxwell, Lewis S. Technical Representative Pesticide Division of Jackson Grain Company, Tampa, Fla.

May, Curtis. United States Department of Agriculture, Bureau of Plant Industry, Beltsville, Md. (Forest pathology.)

McCallan, S. E. A. Plant Pathologist, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Fungicides.)

McClain, R. L. Associate Plant Pathologist, Bureau of Plant Pathology, California Department of Agriculture, P. O. Box 1066, Riverside, Calif. (Citrus diseases.)

McClellan, W. D. Director of Research, Mid-State Chemical Supply Company, P. O. Box 586, Lindsay, Calif.

McClintock, J. A. Professor of Horticulture, Department of Horticulture, Purdue University, West Lafayette, Ind. (Virology, compatability.)

McClure, Thomas T. 825 Lantern Hill Dr., East Lansing, Mich.

McColloch, Lacy P. Plant Pathologist, Division of Handling, Transportation and Storage of Horticultural Crops, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Diseases of fruits and vegetables after harvest.)

McConnell, Louis J. Plant Quarantine Inspector, United States Department of Agriculture, Philadelphia 6, Pa. (Identification foreign plant pathogens.)

McCormick, W. H. Route 5, Box 624B, Tucson, Ariz.

McCubbin, W. A. (Life) 8014 Glenbrook Rd., Bethesda, Md.

McCulloch, Lucia. (Charter, Life, Emeritus) **Retired.** 1851 Wyoming Ave., Washington, D. C.

McDonald, W. C. Department of Plant Pathology, University of Wisconsin, Madison, Wis.

McDonough, E. S. Professor of Botany, Biology Department, Marquette University, Milwaukee 3, Wis. (Cytology fungi, etiology, diseases of ornamentals.)

McFadden, E. S. Agronomist, United States Department of Agriculture, Department of Agronomy, Texas Agricultural and Mechanical College, College Station, Tex. (Cereal breeding and diseases.)

McFarlane, John S. Geneticist, United States Department of Agriculture, P. O. Box 98, Alisal Branch, Salinas, Calif. (Sugar beet diseases, disease resistance.)

McGahen, J. W. Plant Pathologist, Grasselli Chemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Del. (Antibiotics, agricultural chemicals.)

McGrew, John R. Graduate Student, Department of Botany, University of Maryland, College Park, Md. (Tobacco diseases.)

McIntosh, David L. Plant Pathologist, Canada Laboratory of Plant Pathology, Summerland, British Columbia, Canada.

McKeen, Colin D. Associate Plant Pathologist, Plant Pathology Section, Science Service Laboratory, Harrow, Ontario, Canada. (Vegetable diseases.)

McKeen, W. E. Plant Pathologist, Dominion Laboratory of Plant Pathology, Saanichton, British Columbia, Canada. (Root rot and cane gall.)

McKelvey, J. J. Rockefeller Foundation, 49 W. 49th St., New York, N. Y.

McKenzie, Malcolm A. Director of Shade Tree Laboratories, University of Massachusetts, Amherst, Mass. (Forest pathology.)

McKinney, H. H. (Life) Senior Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Virus diseases, virology.)

McLarty, Harold Ross. Officer-in-Charge, Laboratory of Plant Pathology, Summerland, British Columbia, Canada. (Diseases of tree fruits.)

McLaughlin, G. A. Specialist, Seed, Fertilizer, Spray Department, Merrimack Farmers' Exchange, Inc., Concord, New Hampshire. (Fungicides.)

McLaughlin, J. Harvey. Principal Pathologist, Agricultural Division, Battelle Memorial Institute, Columbus 1, Ohio. (Disease loss measurement, epidemiology, tropical plant pathology.)

McLean, Darrell M. Associate Plant Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Vegetable Crops and Diseases, Cooperating with the Northwestern Washington Experiment Station, Mount Vernon, Washington. (Vegetable seed crops.)

McLean, John Grim. Horticulturist, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering, Vegetable Crops and Diseases, United States Department of Agriculture, Aberdeen, Idaho, Branch Experiment Station, Aberdeen, Idaho. (Potato breeding and seed production, disease resistance and breeding for disease resistance, Verticillium wilt, scab, leaf roll.)

McManus, P. J. Manager, Pesticides and Farm Chemicals Department, Grange League Federation Soil Building Service, Division of Cooperative Grange League Federation Exchange, Inc., Ithaca, N. Y. (Fungicides, fruit, vegetable, cereal and forage crop diseases.)

McNabb, Harold S., Jr. Assistant Professor of Forest Pathology, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Diseases of forest trees, wood decay.)

McNew, George L. Managing Director, Boyce Thompson Institute for Plant Research, Inc., 1086 North Broadway,

Yonkers, 3, N. Y. (Fungicides, bacterial diseases, physiology of parasitism.)

McWhorter, Frank P. Plant Pathologist, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Viruses of bulb and vegetable crops, cytology of virus diseases.)

Mehrlich, F. P. IBEC Research Institute, 30 Rockefeller Plaza, Room 5101, New York 20, N. Y.

Meier, Mrs. F. C. (Life) 6402 Beechwood Dr., Chevy Chase, Md.

Meinecke, Emilio P. (Life) Retired Principal Pathologist, United States Department of Agriculture, 3157 Jackson St., San Francisco 15, Calif. (Forest pathology.)

Meiners, Jack P. Assistant Professor of Plant Pathology and Assistant Plant Pathologist, Department of Plant Pathology, Washington State College, Pullman, Wash. (Cereal, forage, and turf diseases.)

Melander, L. W. (Life) Pathologist, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Region V, Minneapolis 1, Minn. (Stem rust control, identification and herbicidal eradication of alternate hosts.)

Melchers, Leo E. Professor of Botany (Plant Pathologist), Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Cereal diseases.)

Melhus, Irving E. (Life) Director Iowa State College, Guatemala Tropical Research Center, Antigua, Guatemala, 407 Pearson Ave., Ames, Iowa. (Plant pathology and tropical plant exploration.)

Menon, Sankara K. Research Assistant and Graduate Student, Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College, College Station, Tex. (Soil-borne diseases.)

Menzies, James D. Plant Pathologist, Bureau of Plant Industry, United States Department of Agriculture, Irrigation Experiment Station, Prosser, Wash. (Soil-borne diseases, virology.)

Merek, Edward L. Graduate Student, Department of Botany, Pennsylvania State College, State College, Pa. (Tree diseases.)

Meuli, Lloyd J. Plant Pathologist, The Dow Chemical Company, Western Division, P. O. Box 245, Seal Beach, Calif. (Fungicides.)

Meyer, Bernard S. Professor and Chairman, Department of Botany and Plant Pathology, Ohio State University, Columbus, and Ohio Agricultural Experiment Station, Wooster; Columbus 10, Ohio. (Plant physiology.)

Meyers, Samuel P. Research Aide, Marine Laboratory, University of Miami, Coral Gables, Fla. (Marine fungi, deterioration of materials.)

Michaelson, Merle E. Assistant Professor of Botany, Department of Botany, University of Missouri, Columbia, Mo. (Cereal diseases, forage crop diseases.)

Middleton, John T. Plant Pathologist, Department of Plant Pathology, University of California, Riverside, Calif. (Vegetable diseases, biology of root rotting fungi, taxonomy of the Pythiaceae.)

Mielke, James L. Pathologist, Division of Forest Pathology, United States Department of Agriculture, Utah State Agricultural College, Forestry Building, Logan, Utah. (General forest pathology.)

Milbrath, David G. Retired Chief of Bureau of Plant Pathology, California Department of Agriculture. 1410 41st St., Sacramento, Calif. (Microbiology.)

Milbrath, John A. Professor of Plant Pathology, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Potato diseases, stone fruit virus diseases.)

Miles, G. F. 100 East Holly Oak Rd., Wilmington 3, Del.

Millar, Paul H. Chief Inspector Arkansas State Plant Board, Box 1069, Little Rock, Arkansas.

Miller, Harold J. Senior Plant Pathologist, Pennsylvania Salt Manufacturing Company, Box 4388, Chestnut Hill Post Office, Philadelphia 18, Pa. (Fungicides, screening methods, fungus physiology.)

Miller, Howard N. Associate Plant Pathologist, Department of Plant Pathology, Florida Agricultural Experiment Station, Gainesville, Fla. (Diseases of ornamental plants.)

Miller, J. J. Associate Professor of Botany, Department of Botany, McMaster University, Hamilton, Ontario, Canada. (Fungus physiology and genetics.)

Miller, Joseph A. 1 South Centre St., South Orange, N. J.

Miller, Julian H. Chairman Division of Plant Pathology, Department of Plant Pathology, University of Georgia, Athens, Ga. (Mycology, Pyrenomycetes.)

Miller, Lawrence I. Associate Plant Pathologist, Virginia Agricultural Experiment Station, Holland, Va. (Peanut diseases.)

Miller, Lawrence P. Biochemist, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Mechanism of fungicidal action, fungus physiology.)

Miller, P. M. Horticulture Field Laboratory, University of Illinois, Urbana, Ill.

Miller, Paul R. Principal Pathologist in Charge, Plant Disease Survey, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Disease surveys and forecasting.)

Miller, Paul W. Plant Pathologist, United States Department of Agriculture, Oregon State College, Corvallis, Ore. (Strawberry virus diseases, walnut and filbert diseases.)

Miller, Pierre A. Department of Agriculture, 146 Physics-Biology Building, 405 Hilgard Ave., Los Angeles 24, Calif.

Miller, Roy E. President and General Manager, Miller Products Company, Foot of S. W. Caruthers St., Portland 1, Ore. (General fungus diseases, antibiotics.)

Miller, William E. Plant Pathologist, Department of the Army, Camp Detrick, Frederick, Md.

Millerburg, Claus J. W. 261 "H" St., Brawley, Calif. (Soil chemistry and commercial pest control.)

Millikan, D. F. Instructor, Department of Botany, University of Missouri, Columbia, Mo. (Fruit disease, virology.)

Milliman, Loren H. Editor Better Fruit Magazine, 1305 S. W. 12th Ave., Portland 1, Oreg. (Pomological diseases and fungicides.)

Mills, Wilford R. Associate Professor of Plant Pathology, Department of Botany, The Pennsylvania State College, State College, Pennsylvania. (Breeding potatoes for disease resistance, genetics and pathogenicity of Phytophthora infestans.)

Minz, G. Plant Pathologist, Division of Plant Pathology, Agricultural Research Station, Rehovot, Israel. (Field crop diseases, nematodes.)

Mishra, J. N. Plant Pathologist and Professor of Plant Pathology, Bihar Agricultural College, Sabour, Bhagalpur, India. (Cereal and vegetable diseases and fungicides.)

Mitchell, John E. Chief, Biological Branch, "C" Division, Camp Detrick, Frederick, Md.

Mix, A. J. (Life) Professor of Botany, Department of Botany, University of Kansas, Lawrence, Kans. (Taphrinales, fungus physiology.)

Mohamed, Hosni A. Graduate Student (Egypt), Plant Pathology Department, University Farm, University of Minnesota, St. Paul 1, Minn. (Cereal diseases.)

Mollicone, Marilyn Noyes. Housewife. 9 Baldwin St., Livermore Falls, Maine. (Fungi imperfecti, blueberry diseases.)

Monroe, Carl M. Plant Pathologist, Shell Agricultural Laboratory, Box 1531, Modesto, Calif. (Bioassay and field testing of fungicides and bactericides, epidemiology of diseases.)

Mooar, Marie. Plant Pathologist, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Ornamental and muck crop diseases.)

Mook, Paul V. Associate Pathologist, Division of Forest Pathology, United States Department of Agriculture, 360 Prospect St., New Haven 11, Conn. (Forest and shade tree diseases, forest products pathology.)

Moore, E. Leon. Agronomist, United States Department of Agriculture and Research Assistant Professor of Plant Pathology, North Carolina State College, Tobacco Experiment Station, Oxford, N. C. (Tobacco breeding and pathology.)

Moore, George T. (Life) Missouri Botanical Garden, St. Louis 10, Mo.

Moore, J. Duain. Associate Profesosr of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Stone fruit virus diseases, fruit diseases, fungicides.)

Moore, M. B. Instructor in Plant Pathology, University Farm, St. Paul 1, Minn. (Grain crops diseases.)

Moore, William D. (Life) Senior Pathologist, United States Department of Agriculture, 501 S. Andrews Ave., Fort Lauderdale, Fla. (Vegetable diseases.)

Morales, Valcárcel Federico G. Industrial Fabricantes de Insecticidas, Simon Bolivar 163, La Habana, Cuba.

Morel, Georges M. Maitre De Recherches, Laboratoire de Physiologie Végétale, Route de Saint Cyr, Versailles, France. (Fungus physiology, fungicides.)

Morgan, Omar D., Jr. 4320 Apartment 101, Ronalt Dr., College Park, Md.

Morris, H. Elwood. Head, Department of Botany and Bacteriology, Montana Agricultural Experiment Station, Montana State College, Bozeman, Montana. (Plant pathology, plant physiology, viruses of stone fruits.)

Morrison, Lou S. Assistant Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma. (Fruit and vegetable diseases.)

Morton, Donald J. Graduate Student, Department of Botany and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Soybean diseases, microbial antagonism.)

Moseman, John G. Plant Pathologist, North Carolina State College, Raleigh, N. C. (Cereal diseases.)

Mowry, James B. Assistant Professor of Horticulture, University of Illinois, Assistant Professor of Agriculture, Southern Illinois University, Superintendent Illinois Horticultural Experiment Station, Southern Illinois University, Carbondale, Ill. (Breeding fruits for resistance to diseases.)

Muir, William H. Department of Plant Pathology, University of Wisconsin, Madison 6, Wis.

Muller, A. S. Professor of Plant Pathology, Department of Botany, University of Florida, Gainesville, Fla. (Plant disease surveys.)

Mullin, R. S. Plant Pathologist, Virginia Truck Experiment Station, P. O. Box 2160, Norfolk, Va. (Vegetable and cereal diseases.)

Muncie, J. H. Professor (Research) of Plant Pathology, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Potato diseases.)

Munger, George Donald. Principal Plant Pathologist, Battelle Memorial Institute, Columbus, Ohio. (Parasite-host relations.)

Munn, Mancel T. (Emeritus) Emeritus Professor of Seed Investigations, Cornell University, 416 East Duarte Rd., Arcadia, Calif.

Munnecke, Donald E. Instructor of Plant Pathology, University of California, Los Angeles 24, Calif. (Ornamental diseases, chemical soil treatments, fungus physiology.)

Murakishi, Harry H. Associate Plant Pathologist, Department of Plant Pathology, University of Hawaii, Honolulu 14, Hawaii. (Virus and fungus diseases of orchids, ornamentals.)

Murphy, Albert M. Agronomist, Division of Sugar Plants, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Box 826, Twin Falls, Idaho.

Murphy, Hickman C. (Life) Principal Pathologist, United States Department of Agriculture, Agronomy Department, Iowa State College, Ames, Iowa. (Oat diseases.)

Myers, James C., Jr. Graduate Student, Department of Plant Pathology, Michigan State College, East Lansing, Mich. (Turf diseases, fungicides.)

Nagel, Clatus M. Department of Plant Pathology, Agricultural Experiment Station, Brookings, S. Dak.

Nakayana, Roy M. Biology Department, State College, N. Mex.

Nance, Nellie W. Pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Plant pathology.)

Natti, John J. Assistant Professor of Plant Pathology, Division of Plant Pathology, New York State Agricultural Experiment Station, Geneva, N. Y. (Vegetable diseases, virology.)

Neal, David C. Pathologist, Division of Cotton and Other Fiber Crops and Diseases, Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Room 119, Agricultural Center, University Station, Baton Rouge 3, La. (Cotton diesases.)

Neergaard, Paul. Plant Pathologist, Danish State Phytopathological Service, Gersons-vej 13, Copenhagen, Denmark. (Seed-borne fungi, Alternaria, Stemphylium.)

Nelson, Klayton E. Assistant Viticulturist in the Experiment Station, and Lecturer in Viticulture, Department of Viticulture, University of California, Davis, Calif. (Post harvest fruit physiology and pathology.)

Nelson, Paul E. Research Assistant, Division of Plant Pathology, University of California, Berkeley 4, Calif. (Root diseases, pathological anatomy.)

Nelson, Ray. Research Professor in Plant Pathology, Michigan State College, East Lansing, Mich. (Diseases muck crops, ornamentals.)

Nelson, Richard R. Graduate Student, Department of Plant Pathology, University of Minnesota, St. Paul 1, Minn. (Forage diseases, alfalfa leafspots.)

Nesbitt, Robert B., Jr. Deputy Agricultural Commissioner and County Plant Pathologist, Orange County Department of Agriculture, 9846 S. Harbor Blvd., Anaheim, Calif. (Fungicides, virology, biological control.)

Nesom, Rivers Y. Professor of Biology, Head Department of Biological Sciences, Southeastern Louisiana College, Hammond, La. (General.)

Newberger, Edward F. Owner, Custom Spray Service, P. O. Box 952, West Palm Beach, Fla. (Fungicides, pathology in general.)

Newhall, Allan G. (Patron) Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Fungicides, soil treatment, nematology.)

Newton, Margaret. 2392 Beach Dr., Victoria, British Columbia, Canada.

Newton, William. Department of Agriculture, Peradeniya, Ceylon.

Nichols, Carl W. Junior Specialist in Plant Pathology, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Virology, fruit diseases.)

Nichols, Lester P. Assistant Professor of Extension Plant Pathology, Room 203, Buckhout Laboratory, Pennsylvania State College, State College, Pa. (Diseases of ornamental shrubs and floral crops.)

Nickel, J. L. P. O. Box 366, Wasco, Calif.

Niederhauser, John S. Plant Pathologist, Rockefeller Foundation, Calle Londres 45, Mexico, D. F., Mexico. (Diesases of potato, corn, beans.)

Nielson, Lowell W. Associate Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C.

Nielson, Mervin W. Graduate Research Assistant, Department of Entomology, Oregon State College, Corvallis, Ore. (Vector entomology, virology.)

Nienstaedt, Hans. Research Assistant, Department of Genetics, The Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven 4, Conn. (Disease resistance and genetics.)

Nikitin, Alexander A. Director, Agricultural Research, Tennessee Corporation, College Park, Ga. (Fungicides.)

Nitz, Carl G. Seed Technologist and Research Associate, Michael-Leonard Company, 21st St. and Clark, Sioux City 6, Iowa.

Nixon, Walter H. Technical Consultant, Ferry-Morse Seed Company, 98 Zabala Rd., Salinas, Calif.

Nolan, Kenneth G. 94 Gardner St., Noroton Heights, Conn.

Nolla, J. A. B. Mayaguez, Puerto Rico.

Nordlinger, Ernest W. Assistant Editor, Food Processing, Putman Publishing Company, 111 E. Delaware Pl., Chicago 11, Ill. (Vegetable and fruit diseases, fungicides.)

Norton, Don C. Assistant Professor of Plant Pathology, Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College, College Station, Tex. (Root rots, soil fungi.)

Norton, Robert J. Associate Director, Crop Protection Institute at Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Insecticides, insecticide resistance, fungicides.)

Nour-Eldin, Farid. 2301 Ashley Ave., Berkeley 5, Calif.

Nugent, Thomas J. Associate Plant Pathologist, Virginia Truck Experiment Station, P. O. Box 2160, Norfolk, Va. (Diseases of vegetable crops.)

Nusbaum, Charles J. Professor of Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Tobacco diseases.)

Nutile, Gabriel E. Chief Seed Analyst, Department of Breeding and Research, Associate Seed Growers, Inc., P. O. Box 406, New Haven, Conn. (Seed testing, physiology of seeds.)

Nyland, George. Assistant Profesosr of Plant Pathology, and Assistant Plant Pathologist, University of California, Davis, Calif. (Virology, mycology.)

O'Brien, Muriel J. Assistant Pathologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Plant Industry Station, Beltsville, Md. (Disease forecasting and distribution.)

Ogawa, Joseph M. Graduate Student, Department of Plant Pathology, University of California, Davis, Calif. (Tree diseases, fungus physiology.)

Olive, Edgar W. (Charter, Life) Retired. 1704 Rodman St., Hollywood, Fla. (Plant diseases, antibiotics.)

Olmsted, Donald S. Graduate Student, Department of Plant Pathology, University of Minnesota, St. Paul 1, Minn. (Potato and ornamental diseases, fungicides.)

Olson, Conrad J. Plant Pathologist, Yoder Brothers, Inc., Barberton, Ohio. (Chrysanthemum diseases.)

Olson, Edward O. Associate Plant Pathologist, United States Department of Agriculture, Citrus Rootstock Investigations, Box 144, Weslaco, Tex. (Citrus rootstocks and diseases.)

Oort, A. Joan P. Professor of Plant Pathology, Director of Laboratory of Plant Pathology, Agricultural University, Binnenhaven 4, Wageningen, Netherlands. (Cereal diseases, late blight of potato, ergot.)

O'Reilly, Henry James. Extension Plant Pathologist, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Fruit diseases, viruses of stone fruits.)

Orellana, R. G. Plant Pathologist, Inter-American Institute of Agricultural Sciences, P. O. Box 24, Turrialba, Costa Rica. (Diseases of potatoes, tobacco, cacao.)

Orsenigo, Mario. Graduate Assistant, University of Arkansas, Fayetteville, Ark.

Ortega, Benjamin C. Ingeniero Agrónomo, Delegación de Defensa Agrícola, Edificio del Banco Ejidal, Morelos 922 Poniente, Torre ón, Coahuila, México. (Cereal and cotton diseases, fungicides.)

Orton, Clayton Roberts. (Life) Professor of Plant Pathology, West Virginia University, Morgantown, W. Va. (Mycology, seed-borne parasites.)

Osmun, A. Vincent. (Life) Emeritus Professor of Botany, University of Massachusetts. Shade Tree Laboratories, University of Massachusetts, Amherst, Mass.

Oswald, John W. Associate Professor of Plant Pathology, Department of Plant Pathology, University Farm, Davis, Calif. (Potato diseases, cereal diseases, virology.)

Oteifa, Bakir A. Plant Pathology Department, University of Maryland, College Park, Md.

Ou, Shu Huang. Senior Specialist, Joint Commission on Rural Reconstruction, 9 Paoching Rd., Taipei, Taiwan, China. (Vegetable, rice, sugarcane diseases.)

Owen, Emily M. Microbiologist, Research and Development Department, Colgate-Palmolive-Peet Company, Jersey City, N. J. (Flora of skin and mouth, industrial contamination problems.)

Owen, Frank C. R. D. 2, Newark, Del.

Owen, John H. Associate Professor of Plant Pathology, Department of Plant Pathology, University of Florida, Gainesville, Fla. (Vegetable diseases, fungicides, mycology.)

Owens, Charles E. Professor Emeritus of Botany and Plant Pathology and Plant Pathologist, Oregon State College, 303 N. 32nd St., Corvalis, Ore. (Fungus diseases of forest trees and agricultural crops, antibiotics, plant viruses.)

Owens, Robert G. Plant Pathologist, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Fungicides, enzymology, fungus physiology.)

Paddock, William C. Professor of Botany, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa, and Director of the Iowa State College-Guatemala Tropical Research Center, 5a Avenida N. No 39, Antigua, Guatemala. (Diseases of tropical crop plants, cereal diseases.)

Padmanabhan, S. Y. Mycologist, Central Rice Research Institute, Cuttack 4, Orissa, India. (Rice diseases.)

Pady, Stuart M. Professor of Botany, Head of Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Mycology, aerobiology, cereal diseases.)

Paharia, Kishan D. Research Assistant, Division of Plant Pathology, University of Minnesota, St. Paul, Minn.

Palm, Elmer Thurman. Fellow in Plant Pathology, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Physiology.)

Palmer, Hugh C. Plant Pathologist, Hercules Powder Company, Wilmington 99, Del. (Fungicides.)

Palmiter, DeForest H. Professor of Plant Pathology, New York State Agricultural Experiment Station, Cottage Road, Poughkeepsie, N. Y. (Fruit diseases, fungicides.)

Panasenko, Vasil T. Professor of Plant Pathology, 340 17th Ave., San Francisco 21, Calif. (Fungus diseases of cereal crops and sugar beets.)

Pantidou, Maria E. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Fungus diseases.)

Panzer, James D. Research Assistant, Department of Plant Pathology, Ohio State Agricultural Experiment Station and Ohio State University, Columbus, Ohio. (Virology.)

Papavizas, George C. Graduate Student, Division of Plant Pathology, University Farm, St. Paul 1, Minn. (Cereal diseases.)

Park, Jai-Young F. Professor of Plant Pathology, Department of Botany and Plant Pathology, Araneta Insti-

tute of Agriculture, Rizal Ave. Extension, Manila, Philippines. (Cereal diseases, mycology.)

Parker, E. K. Naval Research Branch Office, 346 Broadway, New York 13, N. Y.

Parker, Kenneth G. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Tree fruit diseases.)

Parmeter, John R., Jr. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Forest pathology.)

Parris, G. K. Professor of Botany and Head, Department of Botany, Mississippi State College, and Plant Pathologist, State Plant Board of Mississippi, State College, Miss. (Teaching plant pathology, fungicides, breeding for disease resistance.)

Pate, James B. Research Agronomist, Everglades Experiment Station, Belle Glade, Fla. (Plant breeding.)

Patterson, Fred L. Associate Professor of Agronomy, Department of Agronomy, Purdue Universtiy, Lafayette, Ind. (Cereal diseases.)

Patton, Robert F. Assistant Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Forest pathology, tree diseases, forest genetics.)

Paulus, Albert O. Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin.

Pearson, O. H. Plant Breeder, Eastern States Farmers Exchange, Plant Industry Center, South West Street, Feeding Hills, Mass. (Vegetable crop, field corn.)

Peek, Chester A. 819 University Ave., Laramie, Wyo.

Peet, Clyde E. Camp Detrick, Frederick, Md.

Peklo, Jaroslav. Professor Emeritus, Plant Pathology, College of Agriculture and Forestry, Prague, Prague XII, Chodska 3II, Czechoslovakia.

Pelletier, Réal L. Research Assistant in Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, fungus phyisology.)

Peltier, George L. (Emeritus) Emeritus Professor of Bacteriology, University of Nebraska; Consultant, Cranberry Growers, Inc., Wisconsin Rapids, Wis. (Cranberry diseases.)

Perez Otero, J. Enrique. Associate Bacteriologist, Agricultural Experiment Station, University of Puerto Rico, Rio Piedras, Puerto Rico.

Perrault, Champlain J. Officer-in-Charge, Plant Pathology Section, Science Service Laboratory, Ste-Anne-de-la-Pocatière, P. Quebec, Canada. (Potato diseases.)

Person, Lee H. Plant Pathologist, United States Department of Agriculture, North Carolina State College, Box 5397, Raleigh, N. C.

Persons, Thomas D. Plant Pathological Inspector, Phony Peach and Peach Mosaic Disease Control, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 590 Mulberry St., Macon, Ga.

Petersen, Donald H. Associate Pathologist, Fruit and Nut Crops and Diseases, Bureau Plant Industry, United States Department of Agriculture, Department of Botany and Bacteriology, Clemson Agricultural College, Clemson, S. C. (Deciduous fruit tree diseases.)

Peterson, Lester C. Associate Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Potato diseases, breeding.)

Peterson, Paul D. Technical Director Agricultural Sales, Stauffer Chemical Company, Apopka, Fla. (Fungicides, insecticides.)

Peturson, B. Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, Canada.

Phillips, Ruth P. Seed Technologist, Louisiana State Department of Agriculture, Baton Rouge, La. (Germination problems.)

Pierce, Walter H. Plant Pathologist, Associated Seed Growers, Inc., Route 3, Twin Falls, Idaho. (Vegetable diseases, disease resistance in vegetables.)

Pieringer, Arthur P. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Potato diseases, breeding for disease resistance.)

Piersma, Sidney F. Plant Pathologist, California Packing Corporation, Rochelle, Ill. (Cereal diseases and legume diseases, fungicides.)

Pierson, Charles F. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, disease resistance.)

Piesbergen, N. Entomologist, 248 Summer Blvd., Collinsville, Ill.

Pilgrim, A. J. Graduate Assistant, Department of Plant Physiology and Pathology, Agricultural and Mechanical College, College Station, Tex.

Pinckard, J. A. Plant Pathologist, Julius Hyman & Company Division Shell Chemical Corporation, P. O. Box 2171, Denver 1, Colo. (Fungicides.)

Pine, Thomas S. University of California, Davis, Calif.

Pirone, Pascal P. Plant Pathologist, The New York Botanical Garden, Bronx Park, New York 58, N. Y. (Diseases ornamental plants and trees, air pollution.)

Plakidas, A. G. Professor of Botany and Plant Pathologist, Louisiana State University, Baton Rouge 3, La. (Diseases of ornamental plants.)

Plunkett, Orda A. Professor of Botany, Department of Botany, University of California at Los Angeles, Los Angeles 24, Calif. (Fungus physiology, medical mycology.)

Poland, George L. In Charge, Dietetic and Laboratory Research, United Fruit Company, Pier 3, North River, New York 6, N. Y. (Fungicides.)

Pollard, Ernest C. Professor Biophysics, Sloane Physics Laboratory, Yale University, New Haven, Conn. (Physical studies of viruses, radiation biology.)

Pomerleau, Rene. Officer-in-Charge, Laboratory of Forest Pathology, Canada Department of Agriculture, Faculty of Forestry, Laval University, Quebec, Quebec, Canada. (Tree diseases.)

Pon, Dick S. Plant Pathologist, Camp Detrick, Frederick, Md. (Phytopathology.)

Pontis-Videla, Rafael E. Senior Plant Pathologist, Division of Plant Pathology, Instituto Nacional De Agricultura, Maracay, Venezuela. (Vegetable diseases, virology.)

Pool, R. J. (Emeritus) University of Nebraska, Lincoln 8, Nebr.

Poole, D. Donald. Agent (Pathologist), United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Bureau Tobacco, Medicinal and Special Crops, stationed at Texas Agricultural Experiment Substation No. 12, Chillicothe, Tex. (Oil crops pathology, plant breeding.)

Poole, Robert Franklin. President of The Clemson Agricultural College, Clemson, S. C. (Formerly Professor and Plant Pathologist.)

Popp, W. Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, P. O. Box 322, University of Manitoba, Winnipeg, Manitoba, Canada. (Cereal smuts.)

Porte, William S. Plant Pathologist, Division of Vegetable Crops and Diseases, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Vegetable diseases, breeding vegetable plants resistant to diseases.)

Porter, Charles L. Professor of Plant Sciences, Department of Biological Sciences, Purdue University, West Lafayette, Ind. (Industrial mycology, fungus physiology.)

Porter, Clark A. Associate Plant Pathologist, Boyce Thompson Institute for Plant Research, Inc., 1086 N. Broadway, Yonkers 3, N. Y. (Virology.)

Porter, Donald R. (Life) Plant Pathologist and Geneticist, Campbell Soup Company Research Farm, Davis, Calif. (Vegetable breeding and pathology.)

Porter, Frank M. Graduate Research Assistant, Department of Plant Pathology, University of Delaware, Newark, Del.

Porter, Harold L. Horticulture Inspection Supervisor, Ohio Department of Agriculture, Columbus 15, Ohio. (Fruit, forest and ornamental diseases.)

Porter, John N. Research Mycologist, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. (Antibiotics, mold fermentations.)

Porter, R. Howard. Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, and Plant Pathologist Agricultural Experiment Station, Colorado Agricultural and Mechanical College, Fort Collins, Colo. (Cereal diseases, seed-borne organisms.)

Porter, Richard P. Product Development Department, Ethyl Corporation, 100 Park Ave., New York 17, N. Y. (Development of agricultural chemicals.)

Postigo, Rosendo. Escuela Nacional di Agriculture, La Molina, Lima, Peru.

Poulos, Panos L. Plant Pathologist, E. I. du Pont de Nemours & Co., Grasselli Chemicals Department, Room 860, Broad St. Station Building, Philadelphia 3, Pa. (Fungicides.)

Pound, Glenn S. Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, virology.)

Povah, Alfred H. (Life) Science Teacher, Edwin Denby High School, 12800 Kelly Rd., Detroit 21, Mich. (Mycology.)

Powell, Dwight. Associate Professor of Plant Pathology, University of Illinois, Horticulture Field Laboratory, Urbana, Ill. (Fruit diseases, fungicides.)

Powers, Harry R., Jr. Graduate Student, Plant Pathology, North Carolina State College, Box 5397, Raleigh, N. C. (Physiology of parasitism.)

Powers, William J. Instructor Adult Education, Atlantic County Vocational Schools, Minotola, N. J. (Fruit, vegetables, flowers.)

Prasad, Nirankar. Professor of Plant Pathology and Bacteriology, Agricultural Institute, Anand, Gujerat, India. (Plant diseases caused by genus Fusarium; herbicides and teaching.)

Pratt, Robert M. Associate Entomologist-Pathologist, University of Florida, Citrus Experiment Station, Lake Alfred, Fla. (Fruit diseases, ecological aspects of disease and insect control, spraying equipment.)

Presley, John T. Plant Pathologist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Diseases of cotton and other fiber groups.)

Price, W. C. Professor of Botany, Director of the Plant Virus Laboratory, Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pa. (Virology.)

Prielipp, Donald Otto. Forest Pathologist, William Bonifas Lumber Company, Iron Mountain, Mich. (Forest diseases and their control.)

Prince, Alton E. Chief, Biochemical Section, Materials Laboratory, Wright-Patterson Air Force Base, 612 Phillips St., Yellow Springs, Ohio. (Prevention of deterioration by fungi of air force materials.)

Pristou, Robert. Graduate Assistant, Department of Plant Pathology, West Virginia University, Morgantown, W. Va. (Vegetable pathology.)

Pryor, Dean E. Pryor Farms, P. O. Box 756, Gonzales, Calif. (Vegetable pathology.)

Pulsifer, Herbert G. Graduate Student, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Root rots.)

Purdy, L. H., Jr. College of Agriculture, University of California, Davis, Calif.

Putnam, Henry N. Project Leader, White Pine Blister Rust Control, United States Bureau of Entomology and Plant Quarantine, Region V, 301 Metropolitan Building, Minneapolis 1, Minn. (Forest pathology, forest entomology, forest management.)

Raabe, Robert D. Instructor and Junior Plant Pathologist, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Diseases of ornamentals and Armillaria and Dematophora root rots.)

Racicot, H. N. Plant Pathologist, Division of Botany and Plant Pathology, Science Service, Carling Avenue, Ottawa, Ontario, Canada.

Radebaugh, A. D. Blue Mountain Canneries, Dayton, Wash.

Rader, Wm. E. Plant Pathologist, Shell Agricultural Laboratory, Box 1531, Modesto, Calif. (Fungicides, fungus physiology.)

Raeder, J. Milford. Associate Professor, Associate Plant Pathologist, Department of Plant Pathology, University of Idaho, Moscow, Idaho. (Field crop diseases.)

Raleigh, Walter P. Farmer, Clyde, Kans. (Potato diseases.)

Ram, C. S. Venkata. Graduate Student, University of Minnesota, 1421 Cleveland Ave., St. Paul, Minn.

Ramsey, Glen B. Principal Pathologist, United States Department of Agriculture, 5630 Ingleside Ave., Chicago 37, Ill. (Market diseases of fruits and vegetables.)

Rands, Robert D. (Emeritus) Retired Head, Division of Rubber Plant Investigations, United States Department of Agriculture, Lake Wales, Fla.

Rankin, Harvey W. Georgia Coastal Plain Experiment Station, Tifton, Ga.

Rankin, W. H. (Life) Bureau of Plant Industry, New York State Department of Agriculture and Markets, Albany, N. Y.

Raski, Dewey J. Assistant Nematologist, Department of Entomology and Parasitology, University of California, Berkeley 4, Calif. (Plant parasitic nematodes.)

Rawlins, Thomas E. (Life) Professor of Plant Pathology and Plant Pathologist in the Experiment Station, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Virus diseases, plant histochemistry.)

Ray, W. Winfield. Professor of Botany and Mycologist in the Experiment Station, Department of Botany, University of Nebraska, Lincoln 8, Nebr. (Diseases of grasses, morphology and taxonomy of fungi.)

Raychaudhuri, S. P. Indian Agricultural Research Institute, Mycology and Plant Pathology, New Delhi, India.

Reddick, Donald. (Charter, Life) Emeritus Professor of Plant Pathology, Cornell University, R. D. 5, Gainesville, Fla. (General pathology.)

Reddy, Charles S. Professor of Plant Pathology, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Cereal diseases, fungicides, seed treatment.)

Reed, George M. (Charter, Life) Curator Emeritus, Plant Pathology, Brooklyn Botanic Garden, N. Y. 25 S. Linwood Ave., Pittsburgh 5, Pa. (Cereal diseases, physiological races of smuts of oats, wheat, and barley.)

Reed, Howard E. Associate Research Plant Pathologist, University of Tennessee Agricultural Experiment Station, Knoxville, Tenn. (Cereal diseases, cotton, tobacco, forage crops.)

Reed, T. Walter. Assistant Manager Research and Development, (Eastern), California Spray Chemical Corporation, Haddonfield, N. J. (Fungicides.)

Reeves, E. L. Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Box 99, Wenatchee, Wash. (Virus and other diseases of deciduous fruits.)

Reigelman, Gordon L. Research Assistant, Graduate Student, Department of Botany and Plant Pathology, The Ohio State University, Columbus 10, Ohio. (Fungicides.)

Reilly, Joseph J. Division of Plant Pathology, University Farm, St. Paul 1, Minn.

Reinking, Otto A. Professor Emeritus of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, N. Y. Foreign Agricultural

Advisor, Office Foreign Agricultural Relations, United States Department of Agriculture, Washington 25, D. C.

Remy, R. H. Compania Vinicola di Saltidio, Xicotancatl Nte 704, Saltillo, Coah, Mexico.

Rettew, G. Raymond. Managing Director, Antibiotic Laboratories, Wyeth Pharmaceuticals, Inc., West Chester, Pa. (Antibiotics, cultivated mushroom industry consultant.)

Rex, Edgar G. Plant Pathologist, New Jersey Department of Agriculture, Post Office Box 1, Trenton, N. J. (Shade and forest tree pathology.)

Reynard, George B. Plant Pathologist and Geneticist, Agricultural Research Department, Campbell Soup Company, Riverton, N. J. (Vegetable disease resistance and its inheritance.)

Reynolds, Harold W. United States Field Station, Sacaton, Ariz.

Rhoads, Arthur S. (Life) 29 W. 21st St., Jacksonville 6, Fla.

Rice, William N. 143 St. Johns Road, Rangoon, Burma, India.

Rich, Avery E. Associate Professor of Botany and Plant Pathologist, University of New Hampshire, Durham, N. H. (Fungicides, virology, potato diseases, apple scab.)

Rich, Saul. Associate Pathologist, Department of Plant Pathology, The Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven 4, Conn. (Fungicides, fungus physiology, vegetable diseases.)

Richards, B. L. Professor of Plant Pathology, Department of Botany and Plant Pathology, Utah State Agricultural College, Logan, Utah. (Virus diseases.)

Richards, B. Lorin, Jr. Plant Pathologist, Agricultural Chemical Section, Grasselli Chemical Department, E. I. du Pont de Nemours & Co., Experimental Station, Wilmington, Del.

Richards, Mathias C. Professor of Botany, Associate Dean College of Agriculture and Associate Director of Agricultural Experiment Station, University of New Hampshire, Durham, N. H. (Mycology, fungicides.)

Rieman, Gustav H. Professor of Genetics and Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, disease resistance.)

Riker, A. J. (Life) Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Pathological growth, bacterial diseases, diseases of forest trees, research methods.)

Ringel, Samuel M. Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich.

Ritchie, Calvin L. Area Supervisor, United States Bureau of Entomology and Plant Quarantine, Interior District, Memphis 3, Tenn.

Roane, Curtis W. Associate Plant Pathologist, Virginia Agricultural Experiment Station, Blacksburg, Va. (Cereal pathology, fungus genetics, cytology.)

Robert, Alice L. Plant Pathologist, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Cereal diseases, corn.)

Roberts, Bill J. Graduate Student, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Cereal diseases.)

Roberts, Daniel A. Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Diseases of forage crops, teaching.)

Roberts, John W. (Charter, Life) Retired Principal Pathologist, United States Department of Agriculture, Box 926, Winter Park, Fla. (Fruit diseases, fungicides.)

Robles Gutierrez, Leonel. Institue Technologico y de Estudios Superiores de Monterrey, Monterrey, N. L. Mexico.

Rochow, William F. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Virology.)

Rodenhiser, Herman A. Head, Division of Cereal Crops and Diseases, Plant Industry Station, Beltsville, Md. (Cereal diseases.)

Rodriguez Landaeta, Alejandro, Ingeniero Agrónomo, Profesor de Fitopatología en la Facultad de Ingeniería Agronómica y Jefe de la División de Fitapatología, División de Fitopatología, Apartado 669, Maracay, Venezuela. (Phytopathology of seeds and epidemiology.)

Rogers, C. Hoyt. Plant Pathologist, Coker's Pedigreed Seed Company, Hartsville, S. C. (Tobacco and cotton breeding, field crop diseases, farming.)

Rogers, Virginia. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Histology and physiology of fungus diseases, entomology.)

Rogerson, Clark T. Mycologist and Assistant Professor of Botany, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Fungus taxonomy, parasitic fungi of Kansas.)

Roll-Hansen, Finn. Associate Professor of Forest Botany, The Agricultural College of Norway, Vollebekk, Norway. (Forest pathology.)

Rorer, James Birch. (Charter, Life) Manager, Compañia Agricola, San Miguel, Milagro, Ecuador, South America. (Diseases of tropical crops.)

Rosberg, David W. Assistant Professor, Department of Plant physiology and Pathology, Texas Agricultural and Mechanical College, College Station, Texas. (Fruit and vegetable diseases, virology.)

Rose, Raymond C. Extension Plant Pathologist, Agricultural Extension Service, Institute of Agriculture, University of Minnesota, St. Paul 1, Minn. (Extension work in disease control.)

Rosen, H. R. Professor of Plant Pathology, Department of Plant Pathology, University of Arkansas, Fayetteville, Ark. (Breeding small grains and roses for disease resistance, cereal diseases.)

Rosenberg, Dan Y. Junior Plant Pathologist, California Department of Agriculture, Bureau of Plant Pathology, P. O. Box 1066, Riverside, Calif. (Stone fruit and citrus fungus and virus diseases.)

Ross, A. Frank. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Virology.)

Ross, Arnold F. Plant Pathology Department, University of Wisconsin, Madison, Wis.

Ross, John P. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Rossetti, Victoria. Plant Pathologist, Secção de Fitopatologia, Instituto Biologico. C. P. 7119, São Paulo, Brazil. (Fungus physiology, citrus and avocado diseases.)

Rossouw, D. J. Kroondal Tobacco Research Station, P. O. Rustenburg, Transvaal, Union of South Africa.

Roth, Elmer R. Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Asheville, N. C. (Forest tree diseases, cankers, rots, wilts.)

Roth, Herbert. Plant Quarantine Inspector, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 209 River St., Hoboken, N. J.

Roth, Lewis F. Associate Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Forest pathology.)

Roure, Luis A. Professsor of Plant Pathology, College of Agriculture and Mechanic Arts, University of Puerto Rico, Mayaguez, Puerto Rico. (Mycology.)

Rowell, John B. Research Associate, Department of Plant Pathology and Botany, University of Minnesota, St Paul 1, Minn. (Physiology of plant disease, fungus physiology and genetics.)

Rudolph, Bert A. Associate Plant Pathologist, In charge of University of California Deciduous Fruit Field Station, 3099 Stevens Creek Road, San Jose, Calif. (General pathology, bacteriology.)

Ruehle, George D. Vice Director in Charge, University of Florida Subtropical Experiment Station, Route 2, Box

508, Homestead, Fla. (Diseases of potato, mango, and avocado.)

Runnels, Harmon A. Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio. (Diseases of ornamental plants, fungicides, antibiotics.)

Rupert, Joseph A. Associate Plant Pathologist, Rockefeller Foundation, Ministry of Agriculture, Bogota, Colombia. (Cereal diseases, genetic resistance.)

Rusden, Philip L. Assistant Professor (Research) Shade Tree Laboratory, University of Massachusetts, Waltham, Field Station, 240 Beaver St., Waltham 54, Mass. (Tree and shrub diseases, fungicides.)

Rushdi, Medhat H. K. Graduate Student, University of Maryland, College Park, Md. (Fungus physiology, antibiotics, control.)

Russell, Ralph C. Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Department of Agriculture, % University of Saskatchewan, Saskatoon, Saskatchewan, Canada. (Cereal smuts and other seed-borne diseases.)

Ryker, Truman C. Plant Pathologist, Grasselli Chemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Del. (Seed treatments.)

Sacco, Paul. Agricultural Director, St. Ambrose College, Davenport, Iowa. (Physiology, nutritional aberrations.)

Sackston, W. E. Plant Pathologist, Laboratory of Plant Pathology, % University of Manitoba, Winnipeg, Manitoba, Canada. (Diseases of oil-seed crops.)

Sakimura, K. Assistant Entomologist, Pineapple Research Institute of Hawaii, P. O. Box 3166, Honolulu 2, Hawaii. (Thrips transmission of plant diseases.)

Sands, D. R. Professor of Botany, Department of Botany, Ontario Agricultural College, Guelph, Canada. (Vegetable diseases.)

Sanford, G. B. Pathologist-in-Charge, Laboratory of Plant Pathology, Science Service, Canada Department of Agriculture, % University of Alberta, Edmonton, Canada. (General.)

Santacroce, Nunzio G. 215 Treadwell Ave., Staten Island 2, N. Y.

Santilli, Vincent. Graduate student, Division of Plant Pathology, University of California, Berkeley 4, Calif. (Obligate parasites.)

Sasscer, E. R. Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D. C.

Sasser, J. N. Assistant Plant Pathologist, Box 5397, North Carolina State College, Raleigh, N. C. (Plant parasitic nematodes.)

Saunders, W. B. Box 699, Hollister, Calif.

Schafer, John F. Associate Pathologist, Department of Botany and Plant Pathology, Purdue University, Lafayette, Ind. (Diseases resistance, cereal disease, cereal breeding.)

Schafer, Lewis A. Assistant Professor of Botany, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Cereal and vegetable diseases.)

Schaller, Charles W. Assistant Professor of Agronomy, Department of Agronomy, University of California, College of Agriculture, Davis, Calif. (Cereal diseases.)

Scheffer, Robert P. Research Associate, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, physiology of parasitism.)

Schein, Richard D. Assistant Plant Pathologist, State Natural History Survey Division, Section of Applied Botany and Plant Pathology, 385 Natural Resources Building, Urbana, Ill. (Oak wilt, stone fruit viruses, fruit tree diseases.)

Schenck, Norman Carl. Graduate Student, Research Assistant, Natural History Survey, Champaign, Ill. (Forest and shade tree diseases.)

Schexnayder, Charles A. Plant Pathologist, United States Sugar Plant Field Station, Box 470, Houma, La.

Schlatter, F. P. Retired. 3118 Newton St., N. E., Washington, D. C.

Schlegel, David E. Graduate Student in Plant Pathology, University of California, Berkeley 4, Calif. (Physiology and chemotherapy of virus diseases.)

Schlocker, Archie. Associate Plant Pathologist, Bureau of Plant Pathology, California Department of Agriculture, Sacramento 14, Calif. (General plant pathology.)

Schmitt, Chris G. Plant Pathologist, Camp Detrick, Frederick, Md. (General phytopathology.)

Schmitthenner, August F. Instructor, Department of Botany and Plant Pathology, Ohio Experiment Station, Wooster, Ohio. (Diseases of forage crops, physiology of parasitism, ecology of soil fungi.)

Schmitz, Henry. (Life) President, University of Washington, Seattle 5, Wash. (Forest pathology, wood preservation.)

Schnathorst, William C. Graduate Student, Botany Department, University of Wyoming, Laramie, Wyo. (Bacterial diseases.)

Schneider, Charles L. Assistant Pathologist, United States Department of Agriculture, Department of Plant Pathology, University Farm, St. Paul 1, Minn. (Sugar beet diseases.)

Schneider, Henry. Assistant Plant Pathologist, Citrus Experiment Station, Department of Plant Pathology, University of California, Riverside, Calif. (Pathological plant anatomy, citrus pathology.)

Schneider, Irving R. Graduate Student Research Assistant, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Virology, chemotheraphy.)

Schoeneweiss, D. F. 469 Tibet Rd., Columbus 2, Ohio.

Schroeder, Harry W. Graduate Student, Department of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minn. (Cereal diseases, genetics of fungi, fungus physiology.)

Schroeder, Wilbur T. Professor of Plant Pathology, Division of Plant Pathology, New York Agricultural Experiment Station, Cornell University, Geneva, N. Y. (Canning crop vegetable diseases, fungicides, disease resistance.)

Schuldt, Paul H. Plant Pathologist, Boyce Thompson Institute for Plant Research Inc., 1086 N. Broadway, Yonkers 3, N. Y. (Fungicides.)

Schultz, Eugene S., Principal Pathologist, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Potato diseases, disease resistance.)

Schuster, Max L. Assistant Plant Pathologist, Department of Plant Pathology, University of Nebraska, Lincoln, Nebr. (Nematodes of plants, rust studies, diseases of sugar beets, safflower, and special crops.)

Schwarze, Carl Alois. (Life) Retired. 181-20 Midland Pkwy., Jamaica Estates, Long Island, N. Y. (Diseases of ornamentals, antibiotics, role of trace elements in plant physiology.)

Schwegmann, Jack C. Graduate Research Assistant, Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Cotton diseases, pathogenicity of Rhizoctonia solani.)

Schwinghamer, Erwin A. Agent, United States Department of Agriculture, Plant Pathologist, State College Station, Fargo, N. Dak. (Cereal diseases.)

Scott, C. Emlen. Extension Plant Pathologist, University of California, Berkeley 4, Calif. (General plant pathology, micronutrients.)

Scott, William W. Teaching Fellow in Botany, Department of Botany, University of Michigan, Ann Arbor, Michigan. (Aquatic Phycomycetes.)

Seal, J. L. Auburn, Ala.

Segall, Ralph H. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. Vegetable diseases.)

Self, Raymond L. Plant Pathologist, Ornamental Horticulture Field Station of Alabama Polytechnic Institute, Spring Hill, Alabama. (Ornamental pathology.)

Seliskar, Carl E. Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colo. (Fruit and shade tree diseases.)

Semeniuk, George. Professor of Plant Pathology, Department of Plant Pathology, South Dakota State College, Brookings, S. Dak. (Diseases of corn cereals, forage legumes, root rots, fungus physiology.)

Sempio, Cesare. Professor of Plant Pathology Istituto di Patologia vegetale, Università di Perugia, Italy. (Physiopathology, resistance mechanisms, endoprevention.)

Senn, Pettus H. Head Profesor of Agronomy, Department ment of Agronomy, University of Florida, Gainesville, Fla. (Field crops, plant breeding.)

Sequeira, Luis. Apartado 678, San Jose, Costa Rica.

Shafer, W. B. 467 Deodora Dr., Mountain View, Calif.

Shands, H. L. Professor of Agronomy, Agronomy Department, University of Wisconsin, Madison 6, Wis.

Shands, R. G. Professor of Agronomy, Agronomy Department, University of Wisconsin, Madison 6, Wis. (Cereal diseases.)

Shanor, Leland. Professor of Botany and Curator Mycological Collections, Department of Botany, University of Illinois, Urbana, Ill. (Mycology in general, especially Phycomycetes, Ascomycetes, and entomogenous fungi, industrial mycology, general plant pathology.)

Sharp, Eugene L. Graduate Student, Plant Pathology, Iowa State College, Ames, Iowa. (Fungus psysiology.)

Shaw, Charles Gardner. Associate Professor and Associate Plant Pathologist, Department of Plant Pathology, The State College of Washington, Pullman, Wash. (Mycology, Peronosporaceae, forest pathology.)

Shay, J. Ralph. Professor of Plant Pathology, Department of Botany and Plant Pathology, Purdue University, Lafayette, Ind. (Fruit pathology.)

Shea, Keith R. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Forest pathology, tree breeding.)

Shear, Cornelius Lott. (Charter, Life) Collaborator-Mycology and Disease Survey, United States Department of Agriculture, 1707 N. Sixth St., Monroe, La. (Fruit and tree diseases, Pyrenomycetes.)

Shear, E. V. (Life) Plant Pathologist, self-employed, Ravena, N. Y. (Fruit and vegetable diseases, fungicides, insecticides.)

Shepard, Calvin E. Plant Pathological Inspector, Phony Peach Disease Control Project, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 590 Mulberry St., Macon, Ga. (Virus diseases of stone fruits.)

Shepherd, H. R. Connecticut Chemical Research Corporation, Railroad and Bostwick Avenues, Bridgeport, Conn.

Sher, Samuel A. Assistant Nematologist, Department of Plant Pathology, University of Hawaii, Honolulu 14, Hawaii. (Plant parasitic nematodes, nematode taxonomy.)

Sherbakoff, C. D. (Emeritus) Retired. Plant Pathologist the University of Tenness, Agricultural Experiment Station, Knoxville, Tenn.

Sherf, Arden F. Assistant Professor of Botany and Extension Plant Pathologist, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Corn, oat, and potato diseases, fungicides, plant disease survey.)

Sherwin, Helen S. Plant Pathologist, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Forage diseases.)

Sherwood, Robert T. Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Forage crop diseases.)

Shields, Ivan J. Extension Specialist in Plant Pathology, Agricultural Extension Service, University of Arizona, Field Station Box 751, 1201 West Madison, Phoenix, Ariz. (Field crops, vegetables, and orchard diseases.)

Showalter, Joseph W. Inactive status, Scientists Cliffs, Port Republic, Md.

Shukla, T. N. %Sri Shiva Mangal Shukla, Office of the Director of College Indus, U. P. Kanpur, India.

Shumard, Roland S. Monsanto Chemical Company, 1700 S. 2nd St., St. Louis 4, Mo.

Shurtleff, Malcolm C., Jr. Assistant Research Professor and Assistant Extension Professor of Plant Pathology and Entomology, Department of Plant Pathology-Entomology, Agricultural Experiment Station, University of Rhode Island, Kingston, Rhode Island. (Teaching plant pathology, fungus physiology.)

Sideris, Christos P. (Life) Box 3166, Honolulu 2, Hawaii.

Siegel, Albert. Research Associate, Department of Botany, University of California, Los Angeles 24, Calif. (Virology.)

Sikora, Berwin R. Assistant Professor of Botany, Quincy College, Quincy, Ill. (General plant pathology.)

Silber, Gustave. Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Silberschmidt, Karl M. Chefe da Secção de Fisiologia Vegetal Instituto Biológico, Caixa Postal 7119, São Paulo, Brazil. (Virus diseases, immunity.)

Sill, Webster H., Jr. Assistant Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans. (Virology, vegetable diseases, cereal diseases.)

Silverborg, Savel B. Research Associate, Department of Forest Botany and Pathology, State University of New York, College of Forestry, Syracuse 10, N. Y. (Forest tree diseases, wood products pathology.)

Simmonds, Prye M. Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Department of Agriculture, % University of Saskatchewan, Saskatoon, Saskatchewan, Canada. (Root diseases of cereals.)

Simmons, Sidney A. Editor (Plant Sciences) G. and C. Merriam Company, 47 Federal St., Springfield, Mass. (Plant pathology, general botany, especially terminology.)

Simons, John N. Research Assistant in Entomology, University of California, Berkeley, Calif.

Simons, Marr D. Agent, United States Department of Agriculture, Botany Hall, Iowa State College, Ames, Iowa. (Cereal diseases.)

Simonsen, Maryhelen. Route 1, Box 440W, Tucson, Ariz.

Simpson, Geddes W. Entomologist, Maine Agricultural Experiment Station, University of Maine, Orono, Maine. (Potato virus diseases.)

Sims, Asa C., Jr. Graduate Student, Department of Botany and Plant Pathology, Ohio State University, Columbus, Ohio. (Plant pathology.)

Sinclair, James B. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Virology, vegetable crop diseases.)

Sinden, James W. Hauser Champignonkulturen AG., Gossau-Zürich, Switzerland. (Mushroom culture and diseases, physiology of fungi.)

Singh, Sheodhan. Graduate Student (India), University of Minnesota, University Farm, St. Paul 1, Minn.

Sisson, Max A. Area Supervisor, Peach Mosaic Control, United States Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Palisade, Colorado. (Virus diseases of fruit trees.)

Skiles, Robert L. Assistant Plant Pathologist, Rockefeller Foundation Agricultural Program in Colombia, Oficina de Investigaciones Especiales, Facultad de Agronomia, Medellin, Columbia. (Improvement of basic food crops.)

Skolko, A. J. Division of Botany and Plant Pathology, Science Service, Canada Agriculture, Ottawa, Ontario, Canada. (Seed-borne diseases.)

Skoog, Harold A. Tobacco Experiment Station, Route 5, Greenville, Tenn.

Skotland, Calvin B. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases.)

Slack, Derald A. Assistant Professor of Plant Pathology, University of Arkansas, Fayetteville, Ark. (Fruit diseases, stone fruit viruses.)

Slade, Martin A., Jr. Research Bacteriologist and Mycologist, Florida Division, Food Machinery and Chemical Corporation, Lakeland, Fla. (Fungicides, fruit and vegetable diseases.)

Slagg, C. M. (Patron) Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kans.

Sleeth, Bailey. Plant Pathologist, Texas Agricultural Experiment Station, Weslaco, Texas. (Citrus diseases, soil fumigation.)

Slipp, Albert W. Assistant Professor of Forestry, Forest, Wildlife and Range Experiment Station, School of Forestry, University of Idaho, Moscow, Idaho. (Ecology of forest fungi, forest pathology, mycology, technical photography.)

Slutz, Russell C. Horticultural Inspector, Ohio Department of Agriculture, Division of Plant Industry, Columbus 15, Ohio. (Ornamental plant diseases, small fruit and stone fruit fungus and virus diseases, special emphasis on control.)

Slykhuis, John T. Plant Pathologist, Dominion Laboratory of Plant Pathology, Lethbridge, Alberta, Canada. (Cereal diseases.)

Small, Cyril G. (Life) County Agricultural Agent, Wayne County Farm and Home Bureau and 4-H Club Association, Alton, N. Y. (Fruit diseases and insects.)

Smith, Albert L. Pathologist Department of Agronomy, Alabama Polytechnic Institute, Agricultural Experiment Station and Division of Cotton and Other Fibre Crops, United States Department of Agriculture, Auburn, Ala. (Cotton diseases.)

Smith, Cecil G. Assistant Plant Breeder, McNair's Yield-Tested Seed Company, Laurinburg, N. C. (Disease resistant varieties.)

Smith, Clayton, O. (Emeritus) Retired. Plant Pathology, University of California Citrus Experiment Station, Riverside, Calif. (Crown gall.)

Smith, David H., Jr. Research Assistant, Division of Plant Pathology, University Farm, St. Paul 1, Minn. (Cereal diseases.)

Smith, Donald J. Plant Pathologist, United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, P. O. Box 909, Brownsville, Tex. (General disease identification.)

Smith, Edward. Pathologist, Bureau of Entomology and Plant Quarantine, Division of Plant Quarantines, 127 United States Court House, El Paso, Tex. (Mycology and pathology.)

Smith, Floyd F. Senior Entomologist, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Agricultural Research Center, Beltsville, Md. (Insect vectors of plant diseases.)

Smith, Glenn E. Graduate Assistant Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio. (Teaching and antibiotics.)

Smith, Harlan E. Extension Plant Pathologist, University of Arkansas, Fayetteville, Ark. (Virus diseases.)

Smith, Harry L. Regional Director, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, 301 Metropolitan Building, Minneapolis 1, Minn. (Insects and plant diseases.)

Smith, Kenneth M. Director, Plant Virus Research Unit, Molteno Institute, Cambridge University, England.

Smith, Marion A. Plant Pathologist, Division of Handling, Transportation and Storage, United States Department of Agriculture, 5630 Ingleside Ave., Chicago 37, Ill. (Market diseases of fruits and vegetables.)

Smith, Oliver F. Research Agronomist, United States Department of Agriculture, Agricultural Experiment Station, University of Nevada, Reno, Nev. (Breeding for disease resistance in alfalfa.)

Smith, Paul G. Associate Professor of Vegetable Crops, Department of Vegetable Crops, University of California, Davis, Calif. (Disease resistance in vegetables.)

Smith, Thomas E. Director of Plant Breeding, McNair's Yield-Tested Seed Company, Laurinburg, N. C. (Tobacco diseases, disease resistance.)

Smith, Wilson L., Jr. Associate Plant Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Division Handling, Transportation and Storage, Beltsville, Md. (Bacterial diseases of potatoes and vegetables, post-harvest fungicides for peaches, antibiotics, potato corking for bacterial rot prevention.)

Smoot, John J. Plant Pathologist, "C" Division, Camp Detrick, Frederick, Md. (Vegetable diseases.)

Smucker, Silas J. Soil Conservationist, United States Conservation Service, Rensselaer, Ind. (Forest tree diseases.)

Snell, Walter H. (Life) Stephen T. Olney Professor of Natural History, Chairman, Department of Botany, Brown University, Providence 12, R. I. (Forest Pathology, mycology-Boletaceae, Hydnaceae.)

Snow, Gordon F. Graduate Research Assistant, Department of Botany, Plant Pathology, Oregon State College, Corvallis, Ore.

Snowden, Parker. Botany Department, Ohio State University, Columbus, Ohio.

Snyder, H. Donald. Mycologist, Microbiologicial Laboratories, Nuodex Products Company, Inc., Elizabeth F, N. J. (Mildew of paints, fungus physiology-morphology, photomicrographic studies.)

Snyder, K. C. Research Assistant, 230 Botanical and Zoological Building, Ohio State University, Columbus, Ohio.

Snyder, William C. Professor of Plant Pathology, University of California, Berkeley 4, Calif. (Vegetable pathology, mycology.)

Solheim, Wilhelm G. Professor of Botany, Department of Botany, University of Wyoming, Laramie, Wyo. (Fungi of the Rocky Mountains, genera of Hyphomycetes.)

Sommer, N. F. Research Assistant, Department of Plant Pathology, University of California, Davis, Calif.

Sowell, Grover, Jr. Graduate Student in Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Vegetable diseases.)

Spaulding, Perley. (Charter, Life) 360 Prospect St., New Haven 11, Conn.

Speairs, Richard K., Jr. Associate Professor of Botany and Forestry, Department of Biology, Centenary College, Shreveport, La. (Forest pathology, diseases of field crops.)

Spencer, Edwin R. Consulting Botanist and Plant Pathologist, Lebanon, Ill. (Air pollution and sick soils.)

Spiegelberg, Carl H. Pathologist, Plant Pathology, Department, Pineapple Research Institute of Hawaii, P. O. Box 3166, Honolulu 2, Hawaii. (Pineapple fruit diseases, canned fruit spoilage, fungus diseases of roots.)

Spilker, Oren W. Assistant Plant Pathologist, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio. (Forest pathology, fungicides.)

Sprague, Roderick. (Life) Plant Pathologist, Tree Fruit Experiment Station, Wenatchee, Wash. (Fruit diseases caused by fungi, leaf spots of Gramineae.)

Sproston, Thomas, Jr. Associate Professor of Botany, Plant Pathologist, Vermont Agricultural Experiment Station, University of Vermont, Burlington, Vermont. (Nature of disease and resistance, Sclerotinia, quinones.)

Staffeldt, Eugene E. Graduate Assistant, Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Plant pathology.)

Stahmann, Mark A. Associate Professor of Biochemistry, Department of Biochemistry, University of Wisconsin, Madison 6, Wis. (Disease resistance, virology, polypeptides and proteins.)

Stakman, E. C. (Life) Head, Department of Plant Pathology and Botany, University of Minnesota, and Agent, United States Department of Agriculture, University Farm, St. Paul 1, Minn. (Physiologic specialization and genetics of plant pathogenic fungi in relation to the development of disease resistant varieties, epidemiology of cereal rusts.)

Stall, Robert E. Graduate Student, Department of Botany and Plant Pathology, Ohio State University, Columbus, Ohio.

Standen, John H. Agricultural Chemicals Development, B. F. Goodrich Chemical Company, Rose Building, Cleveland 15, Ohio.

Stanley, Wendell M. Director, Virus Laboratory, Chairman of the Department of Biochemistry, University of California, Berkeley 4, Calif. (Biochemistry, virology.)

Stanton, W. Robert. Chief Officer, Maize Rust Research Unit, West African Inter-Territorial Secretariat, % Department of Agriculture, Ibadan, Nigeria.

Starcs, Karlis. 5870 Sunset Lane, Indianapolis 20, Ind. (Fungi, Phanerogamae, Bryophyta. Exchange: Bavaria, Ind.)

Stark, Frank L. Group Leader in Charge of Agricultural Research, American Cyanamid Company, Stamford, Conn. (Soil treating agents.)

Starr, George Herman. Director Agricultural Extension Service and Plant Pathologist, University of Wyoming, Laramie, Wyo. (Bacterial diseases of beans and potatoes, antibiotics.)

Starr, Mortimer P. Associate Professor of Bacteriology, Department of Bacteriology, University of California, Davis, Calif. (Metabolism and classification of plant-disease bacteria, general microbiology.)

Statler, Maynard W. Control Supervisor, Bureau of Entomology and Plant Quarantine, Northside Post Office, Box 6479, Pittsburgh 12, Pa. (Regulatory work.)

Steenland, Adin P. Topland Company, Route 2, Prosser, Wash. (Diseases of essential oil producing crops, fungicides.)

Steere, Russell L. Assistant Research Biologist, Virus Laboratory, University of California, Berkeley 4, Calif. (Virology, electron microscopy.)

Steib, René J. Plant Pathologist, Department of Botany, Plant Pathology and Bacteriology, Louisiana State University, Baton Rouge 3, La. (Sugarcane diseases.)

Steinen, Otto P. Representative, United States Rubber Company, Naugatuck Chemical Division, 5901 Anaheim Telegraph Rd., P. O. Box 6818, East Los Angeles, Calif. (Fungicide sales and development.)

Steiner, G. Head Nematologist in Charge, Division of Nematology Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Plant-parasitic, free-living, and insect-parasitic nematodes.)

Steinmetz, Ferdinand H. Head, Department of Botany and Entomology, University of Maine, Orono, Maine. (Fungi of Maine.)

Stessel, George J. Plant Pathologist, Hercules Powder Company, Naval Stores Department, Wilmington, Del. (Fungicides.)

Stevens, Russell B. Associate Professor of Botany, Department of Botany, University of Tennessee, Knoxville 16, Tenn.

Stevenson, F. J. Plant Industry Station, Beltsville, Md.

Stevenson, Frank V. Associate Plant Pathologist, Everglades Experiment Station, Belle Glade, Fla. (Vegetable diseases, fungicides, breeding.)

Stevenson, John A. Head, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md. (Fungus taxonomy and nomenclature.)

Stewart, Dewey. 1736 Webster St., N. E., Washington 17, D. C.

Stewart, Donald M. 300 Agronomy Building, University Farm, St. Paul 1, Minn.

Steyaert, Rene L. 15g Rue Des Atrebates, Etterbeek, Bruxelles IV, Belgium.

Stoddard, David L., Leader, Fungicide Research Group, Agricultural Chemical Section, Grasselli Chemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Del. (Fungicides, nematocides, antibiotics.)

Stoddard, Ernest M. Plant Pathologist, Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, New Haven 4, Conn. (Fruit diseases, chemotherapy.)

Stokes, Granville W. Assistant Plant Pathologist, University of Kentucky, Lexington 29, Ky.

Stoneleigh, Duane. Golf Course Road, Garrison, Md.

Stoner, Warren N. Assistant Plant Pathologist, Everglades Experiment Station, Belle Glade, Fla. (Virology, insect transmitted plant diseases.)

Storey, Harold Haydon. (Life) Deputy Director, East African Agriculture and Forestry Research Organization, Kikuyu, Kenya, East Africa. (Virus diseases.)

Stout, Gilbert L. Plant Pathologist and Chief, Bureau of Plant Pathology, California Department of Agriculture, Sacramento 14, Calif. (Virus diseases of stone fruits, regulatory plant pathology, disease surveys, epidemiology.)

Stover, Robert H. Plant Pathologist, Tropical Research Department, United Fruit Company, La Lima, Honduras, Central America. (Tobacco and banana diseases, soil microbiology in relation to root diseases.)

Stover, W. C. (Life) Department of Botany, Ohio State University, Columbus 10, Ohio.

Stowell, Ewell A. Graduate Student, Department of Botany, University of Wisconsin, Madison 6, Wis. (Fungus morphology, taxonomy.)

Strong, Forrest C. Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Forest pathology, shade tree diseases, oak wilt, damping-off.)

Strong, (Mrs.) Miriam C. Assistant Professor of Plant Pathology (Research), Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Tomato diseases, fungicides, Fusarium wilt.)

Stroube, William H. Graduate Student, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Cereal and forage crop diseases.)

Struble, F. Ben. Associate Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Diseases of fruits and vegetables.)

Suhovecky, Albert J. Graduate Student, Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio. (Fungicides.)

Suit, R. F. Plant Pathologist, University of Florida, Citrus Experiment Station, Lake Alfred, Fla. (Citrus diseases.)

Summer, Eaton M. In Charge, Cane Research, United Fruit Sugar Company, Preston, Oriente, Cuba. (Sugar cane agriculture, virus strains.)

Summers, Thomas E. Everglades Experiment Station, Belle Glade, Fla.

Swaebly, Mary Ann. Division of Plant Pathology, University of Minnesota, St. Paul 1, Minn.

Swank, George, Jr. Assistant Plant Pathologist, Department of Plant Pathology, University of Florida, Agricultural Experiment Stations, Central Florida Experiment Station, Sanford, Fla. (Vegetable diseases, soil fumigants, fungicides.)

Swartwout, Harold G. Associate Professor of Horticulture, Department of Horticulture, University of Missouri, Columbia, Mo. (Fruit crop diseases, chemical control.)

Swenson, Knud G. Assistant Professor of Entomology, New York Agricultural Experiment Station, Geneva, N. Y. (Insect transmission of plant diseases.)

Swingle, John W. Tree Expert, Swingle Tree Surgery Company, 306-7 Enterprise Building, Denver 2, Colo. (Tree diseases and fungicides.)

Swingle, Roger U. Senior Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Post Office Box 70, Worthington, Ohio. (Forest and shade tree diseases, virology, selection and breeding for disease resistance.)

Sylvester, Edward S. Assistant Professor of Entomology, Department of Entomology, University of California, Berkeley 4, Calif. (Virology, insect vectors of plant viruses.)

Szkolnik, Michael. Assistant Professor of Plant Pathology, Division of Plant Pathology, New York State Agricultural Experiment Station (Cornell University), Geneva, N. Y. (Pathology and control of fruit diseases, fungicides, chemotherapy.)

Takahashi, William N. Associate Professor of Plant Pathology and Associate Plant Pathologist, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Virology.)

Takeshita, Robert M. Assistant, Division of Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Ill. (Vegetable diseases, fungus physiology.)

Tammen, James. Graduate Student in Plant Pathology, University of California, Berkeley 4, Calif. (Diseases of ornamentals, taxonomy and physiology of the Fusaria.)

Tanner, Carl R. Research Entomologist, California Spray Chemical Corporation, Box 5946, Portland 22, Ore.

Tapke, Victor F. Plant Pathologist, Plant Industry Station, Beltsville, Md. (Cereal diseases.)

Tarjan, Armen C. Assistant Research Professor of Plant Pathology, Department of Plant Pathology, University of Rhode Island, Kingston, R. I. (Plant parasitic nematodes, ornamental diseases.)

Tartakowsky-Henker, Sergio. Professor of Plant Pathology Universidad de Chile Escuela de Agronomía, Sub-Director del Departamento de Sanidad Vegetal del Ministerio de Agricultura, P. O. B. 4647, Santiago, Chile, South America. (Fruit trees diseases and fungicides.)

Tate, H. Douglas. Manager, Agricultural Chemicals Development, United States Rubber Company, Naugatuck Chemical Division, Bethany 15, Conn.

Taylor, Albert L. Nematologist, Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Md. (Plant parasitic nematodes.)

Taylor, Carlton F. Plant Pathologist, "C" Division, Camp Detrick, Frederick, Md. (Fungicides, diseases of tree fruits.)

Taylor, Gordon S. Assistant Plant Pathologist, The Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Conn. (Vegetable diseases, potatoes, wilt, breeding.)

Taylor, Jack. Assistant Plant Pathologist, Georgia Mountain Experiment Station, Blairsville, Ga. (Fruit and vegetable diseases.)

Tehon, Leo R. Head, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Professor of Plant Pathology, Graduate College, University of Illinois, 385 Natural Resources Building, Urbana, Ill. (Surveys, tree diseases, floricultural diseases, fungicides.)

Ten Houten, J. G. Director, Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 4a, Wageningen, Netherlands. (Evaluation of plant disease losses, plant disease resistance, diseases due to fungi or bacteria.)

Ter Horst, W. P. Mathieson Chemical Corporation, Mathieson Building, Baltimore 3, Md.

Tervet, Ian W. Chief, C Division, Dugway Proving Ground, Tooele, Utah. (Epidemiology.)

Thanos, Andrew. Microbiologist, Department of Antibiotics Manufacturing and Development—K490, Eli Lilly and Company, Indianapolis 6, Ind. (Antibiotics, fungicides, fungus physiology.)

Thayer, Paul L. Graduate Student, Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio. (General.)

Theis, Thomas N. Plant Pathologist, United States Department of Agriculture, Federal Experiment Station, Mayaguez, Puerto Rico. (Cereal diseases, root rots.)

Thirumalachar, M. J. 20th-5th Main Rd., Malleswaram, Bangalore, India.

Thomas, Charles A. Plant Pathologist, Tobacco, Medicinal and Special Crops, Plant Industry Station, Beltsville, Md. (Special crop diseases.)

Thomas, H. Earl. Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Diseases of deciduous fruit trees.)

Thomas, H. Rex. Senior Plant Pathologist, Plant Industry Station, United States Department of Agriculture, Beltsville, Md. (Bean diseases, breeding for disease resistance.)

Thomas, Harold E. Route 2, Box 458, Morgan Hill, Calif.

Thomas, John E. Associate Professor of Botany and Plant Pathology, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Teaching methods, ornamental diseases, tree diseases, general mycology.)

Thomas, Roy C. Assistant Professor, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio. (Bacterial diseases of plants, bacterial viruses and plant viruses.)

Thomas, Samuel O. Research Mycologist, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. (Actinomycetes, antibiotics.)

Thomas, Walter D., Jr. Professor of Plant Pathology and Plant Pathologist, Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colo. (Ornamental, forest and shade tree, and vegetable diseases, fungicides, virology.)

Thompson, George E. Professor of Plant Pathology, Department of Plant Pathology, College Experiment Station, University of Georgia, Athens, Georgia. (General pathology.)

Thornberry, H. H. (Life) Associate Professor of Plant Pathology, University of Illinois, Urbana, Ill. (Virology, biochemistry.)

Thornton, Norwood C. Director of Laboratories, United Fruit Company, Tropical Research Department, La Lima, Honduras. (Fungicides, research and production problems of banana culture.)

Thornton, Raymond L. Plant Pathologist, Wallace & Tiernan Sales Corporation, 1713 S. California Ave., Monrovia, Calif. (Storage rots.)

Thung, T. H. Professor of Plant Virology, Institute for Phytopathological Research, Binnenhaven 4a, Wageningen, Netherlands. (Virology, plant physiology.)

Thurston, H. David. Research and Teaching Assistant in Plant Pathology, Division of Plant Pathology, University of Minnesota, St. Paul 1, Minn. (Potato diseases, vegetable diseases.)

Thurston, Henry W. Jr. Professor of Plant Pathology, Department of Botany, Pennsylvania State College, State College, Pa. (Fungicides, taxonomy, epidemiology of rust fungi.)

Tidd, Joseph S. Plant Pathologist, Department of Breeding and Research, Associated Seed Growers, Inc., P. O. Box 406, New Haven, Conn. (Vegetable crop diseases and their control, disease resistance.)

Tiffany, Lois H. Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa. (Grass diseases.)

Tilford, Paul E. Executive-Secretary, National Arborist Association, Inc., Wooster, Ohio. (Diseases of ornamental plants and shade trees.)

Timian, Roland G. Graduate Student, Horticulture Building, Iowa State College, Ames, Iowa. (Vegetable pathology, virology, especially potato viruses.)

Timonin, Michael I. Director of Research, Pearson and Company, Research Department, Dogwood Lane, Spring Hill, Ala. (Taxonomy and physiology of fungi imperfecti, antagonism and production of antibiotics.)

Tims, Eugene C. Plant Pathologist, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Diseases of truck crops and fruits.)

Tinline, Robert D. Plant Pathologist, Dominion Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. (Cereal diseases.)

Tischler, Nathaniel. Head of Biological Section, Sharples Chemicals, Inc., 317 Leconey Ave., Palmyra, N. J. (Fungicides, bactericides, insecticides, nematocides, plant response chemicals.)

Tisdale, William B. (Life) Head, Division of Botany, Plant Pathologist and Head, Plant Pathology Department, Agricultural Experiment Station, University of Florida, Gainesville, Fla. (Diseases vegetable crops, disease resistance.)

Tisdale, W. H. Room 4156 du Pont Building, Wilmington, Del.

Tochinai, Yoshihiko. Professor of Plant Pathology, Faculty of Agriculture, Hokkaido University, Sapporo, Japan. (Fungus physiology, disease resistance.)

Todd, Edwin H. Plant Pathologist, United States Department of Agriculture, P. O. Box 2967, Beaumont, Texas.

Tomasello, R. P. Box 6156, West Palm Beach, Fla.

Tompkins, C. M. (Life) Department of Plant Pathology, University of California, Berkeley 4, Calif. (Fungus and virus diseases of ornamental and vegetable plants.)

Toole, E. Richard. Pathologist, Division of Forest Pathology, Delta Branch, Southern Forest Experiment Station, Stoneville, Miss. (Forest diseases.)

Torgeson, D. C. Senior Fellow, Boyce Thompson Institute for Plant Research, Inc., 1086 N. Broadway, Yonkers 3, N. Y. (Fungicides.)

Torrey, G. Safford, Professor of Botany, University of Connecticut, Storrs, Conn. (General plant pathology, teaching.)

Townsend, George R. Owner and Director of Florida Field Trials, Box 356, Belle Glade, Fla. (Fungicide trials.)

Treshow, Michael, Senior Laboratory Technician, Graduate Student, University of California, Riverside, Calif. (Vegetable diseases.)

Trkula, David. Graduate Student, Plant Virus Laboratory, University of Pittsburgh, Pittsburgh 13, Pa. (Virology.)

Troutman, Joseph L. Assistant in Plant Pathology, Department of Agronomy, University of Kentucky, Lexington, Ky. (Tobacco diseases.)

Troxel, A. W. Research Assistant, University of California, Riverside, Calif.

True, R. P. Associate Professor of Plant Pathology, Department of Plant Pathology, Bacteriology and Entomology, West Virginia University, Morgantown, W. Va. (Forest pathology, pathological anatomy.)

Trytten, Grover W. Graduate Student, Michigan State College, 812 C Cherry Lane, East Lansing, Mich. (Botany and plant pathology.)

Tsao, Peter H. Graduate Student, Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Antibiotics, fruit diseases.)

Tu, Chih. (Patron) Department of Reconstruction, Tihwa, Sinkiang, China.

Tucker, C. M. Department of Botany, University of Missouri, Columbia, Mo.

Tuite, John F. Graduate Student, Department of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minn. (Fungus physiology, industrial mycology.)

Tullis, Edgar C. Plant Pathologist, "C" Division, Biological Laboratories, Camp Detrick, Frederick, Md. (Rice diseases.)

Turk, Fateh Mohomed. Graduate Student, Division of Plant Pathology, University of Minnesota, St. Paul, Minn. (Forest pathology.)

Turner, Mason M. Shell Agriculture Laboratory, Shell Development Company, P. O. Box 1531, Modesto, Calif.

Twomey, James A. Assistant Plant Pathologist, Washington State Department of Agriculture, Irrigation Experiment Station, Prosser, Wash. (Stone fruit viruses.)

Tyler, L. J. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Teaching, cereal and forage diseases.)

Tyner, L. E. Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, % University of Alberta, Edmonton, Alberta, Canada. (Cereal diseases.)

Ukkelberg, Harry G. Associate Horticulturist, Tidewater Experiment Station, Fleming, Ga.

Ullstrup, Arnold J. Professor of Plant Pathology, Department of Botany and Plant Pathology, Plant and Soils Laboratory, Purdue University, Lafayette, Ind. (Corn diseases.)

Ulman, Paul T. Assistant Entomologist, Department of Conservation, Division of Entomology, 311 W. Washington St., Indianapolis 4, Ind. (Regulatory.)

Utech, Nedra M. 2035 E. Cameron Ave., West Covina, Calif.

Utter, L. Gordon. Plant Pathologist, Diamond Alkali Company, Organic Chemicals Division, 80 Lister Ave., Newark 5, N. J. (Fungicides, disease control.)

Vaartaja, L. Olli. Associate Forest Pathologist, Laboratory of Forest Pathology, 309 Birks Building, Saskatoon, Saskatchewan, Canada. (Forest parasites, ecological factors.)

Valleau, William Dorney. Distinguished Professor of Plant Pathology and Plant Pathologist, Department of Agronomy, University of Kentucky, Lexington, Ky. (Tobacco diseases, breeding for resistance in tobacco.)

Van Arsdel, Eugene P. Research Assistant, Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Forest tree diseases, rusts, climatic factors.)

Van Meter, Mary G. Retired, United States Department of Agriculture, 1316 New Hampshire Ave., N. W., Washington 6, D. C. (Botany, ornithology.)

Vanterpool, T. C. Professor of Plant Pathology, Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. (Diseases of cereals and flax.)

Varney, Eugene H. Plant Pathologist, United States Department of Agriculture, Department of Plant Pathology, Agricultural Experiment Station, New Brunswick, N. J. (Blueberry and cranberry diseases, virology.)

Vasudeva, R. S. Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. (Physiology of parasitism, plant viruses and wheat rusts.)

Vaughan, Edward K. Professor of Plant Pathology, Plant Pathologist, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Diseases of vegetables and small fruits.)

Vaughan, R. E. (Life) R. F. D. No. 1, Ackworth, Iowa.

Vaughn, John R. Associate Professor of Plant Pathology, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich. (Cereal diseases, fungicides, turf diseases.)

Vekemans, Jacques. Plant Pathologist, Institut National pour l'Etude Agronomique du Congo Belge, Division de Phytopathologie, Laboratoire régional de Kaniama, Belgian Congo. (Tobacco.)

Venkatakrishniah, N. S. Assistant Plant Pathologist, Department of Agriculture, Bangalore 1, India. (Cereal diseases and their control.)

Vermillion, M. T. Assistant Professor of Botany, Department of Botany, Ohio University, Athens, Ohio. (Plant nutrition in relation to disease, vegetable diseases.)

Vestal, Edgar F. Plant Pathologist, Office of Foreign Agricultural Relations, Point IV, TCA, American Embassy, Amman, Jordan, % United States Department of State, Washington 25, D. C.

Viennot-Bourgin, G. Institut Nat'l. Agronomique, 16 Rue Claude Bernard, Paris 15e, France.

Virgin, Walter J. Plant Pathologist, California Packing Corporation, 850 Thornton St., San Leandro, Calif. (Vegetable seed and canning crop diseases.)

Visor, Frederick C. Head, Industrial Antibiotic Section, Charles Pfizer Company, Inc., 630 Flushing Ave., Brooklyn, N. Y. (Fungicides, bactericides, antibiotics, plant pathology.)

Vlitos, August John. Senior Fellow, Plant Physiology, Carbide and Carbon Chemicals Company, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Plant growth regulators, photoperiodism.)

Voorhees, R. K. Plant Pathologist and Horticulturist, Wilson and Toomer Fertilizer Company, P. O. Box 3751, Orlando, Fla. (Diseases and insects of citrus, vegetables and pastures, pesticides and fertilizers.)

Wade, Earl K. Assistant Professor of Plant Pathology, College of Agriculture, University of Wisconsin, Madison 6, Wis. (Extension, plant diseases.)

Wadley, Bryce N. Associate Plant Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Utah State Agricultural College, Logan, Utah. (Stone fruit viruses.)

Wadsworth, Dallas F. Assistant Plant Pathologist, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Cereal diseases and virology.)

Wagener, Willis W. Senior Pathologist, Division of Forest Pathology, United States Department of Agriculture, 720 Appraisers Building, San Francisco 11, Calif. (Forest pathology.)

Waggoner, Paul E. Assistant Plant Pathologist, Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, New Haven 4, Conn. (Biophysics, mathematical problems in pathology.)

Wagnon, H. Keith. Assistant Plant Pathologist, Bureau of Plant Pathology, California Department of Agriculture, Sacramento 14, Calif. (Viruses of stone fruits.)

Waldee, E. L. Assistant Agricultural Officer, United States Stem to Indonesia, % State Department Mailing Room, Washington 25, D. C.

Walker, Anson R. Professor of Plant Pathology, Department of Botany, University of Western Ontario, London, Ontario, Canada. (Soil phytopathogens, antibiotics.)

Walker, Earnest A. Chief, Fungicide and Herbicide Section, Insecticide Division, Production and Marketing Administration, United States Department of Agriculture, Washington 25, D. C. (Fungicides, herbicides and disease control.)

Walker, Jerry T. Undergraduate Research Assistant, Department of Botany and Plant Pathology, Ohio State University, Columbus, Ohio. (Fungicides.)

Walker, John C. (Life) Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis. (Vegetable diseases, disease resistance, virology.)

Wallace, Errett. (Charter) Retired Plant Pathologist, United States Department of Agriculture, P. O. Box 415, Purcellville, Loudoun County, Va. (Fungicides.)

Wallace, Henry A. H. Associate Plant Pathologist, Laboratory of Plant Pathology, Science Service, Canada Agriculture, P. O. Box 322, University of Manitoba, Winnipeg, Canada. (Cereal diseases, root rot.)

Wallace, James M. Plant Pathologist, Department of Plant Pathology, University of California, Citrus Experiment Station, Riverside, Calif. (Citrus and avocado diseases, virology.)

Wallin, Jack R. Pathologist, Mycology and Disease Survey, United States Department of Agriculture, Iowa State College, Ames, Iowa. (Bacterial diseases, corn diseases, disease forecasting.)

Walter, James M. Gulf Coast Experiment Station, Bradenton, Fla.

Walters, Hubert J. Assistant Professor of Plant Pathology, Department of Agronomy, University of Wyoming, Laramie, Wyo. (Virology, diseases of beans and cereal crops.)

Warid, Warid A. Plant Breeder, Department of Plant Breeding, Ministry of Agriculture, Orman, Giza, Egypt. (Breeding for disease resistance.)

Warner, Robert M. Research Director, California Fig Institute, Research Laboratory, 5335 N. Palm Ave., Fresno 4, Calif. (Fig canker disease, spoilage diseases of figs.)

Warnock, Stephen J. Green Giant Company, Dayton, Wash. (Breeding for disease resistance.)

Warren, John R. Botany Department, Duke University, Durham, N. C.

Waterhouse, Walter L. (Emeritus) Research Professor in Agriculture, The University of Sydney, Sydney, New South Wales, Australia. (Cereal diseases, general pathology.)

Waterman, Alma M. Pathologist, Division of Forest Pathology, United States Department of Agriculture, 360 Prospect St., New Haven 11, Conn. (Fungus diseases of forest trees, mycology.)

Watkins, Daniel Anthony. Managing Director, Ivon Watkins, Limited, Agricultural Chemicals, 14-20 Buller St., New Plymouth, New Zealand. (Chemotherapeutants and general plant pathology.)

Watkins, G. M. Professor and Head, Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College System, College Station, Tex. (Teaching plant pathology, soil-borne fungal diseases.)

Watson, (Mrs.) Alice J. Associate Mycologist, Mycology and Disease Survey, Plant Industry Station, Beltsville, Md. (Mycology.)

Watson, Roscoe D. Associate Professor of Plant Pathology, Associate Plant Pathologist, Department of Plant Pathology, University of Idaho, Moscow, Idaho. (Vegetable diseases and breeding for disease resistance.)

Watson, William M. Bureau of Entomology and Plant Quarantine, Box 1257, Roanoke 11, Va.

Weathers, Lewis G. Farm Advisor's Office, 52 N. California St., Ventura, Calif. (Vegetable pathology, virology.)

Weaver, Ernest P. Junior Fellow, Mellon Institute of Industrial Research, 4400 Fifth Ave., Pittsburgh 13, Pa. (Fungicides.)

Weaver, L. O. Professor of Plant Pathology, State Plant Pathologist, Department of Botany, University of Maryland, College Park, Md. (Fruit diseases, general extension plant pathology.)

Webb, Raymon E. Associate Horticulturist, Department of Horticulture, Louisiana State University, University Station, Baton Rouge 3, La. (Potatoes, beans and sweet corn.)

Weber, George F. (Life) Professor of Plant Pathology, Department of Plant Pathology, University of Florida,

Gainesville, Fla. (Plant diseases, mycology, tropical deterioration.)

Weber, Paul V. V. Assistant Plant Pathologist and Geneticist, Campbell Soup Company, Farm No. 1, Research Department, Riverton, N. J. (Vegetable diseases, fungicides, breeding disease resistance.)

Weed, Richard M. Assistant Biochemist, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y. (Fungicide mechanisms.)

Wei, C. T. % Mr. Kai Fang, United Mercantile Company, 502 Peddler Building, Hong Kong, China.

Weihing, John L. Extension Plant Pathologist, University of Nebraska, Lincoln 3, Nebr.

Weindling, Richard. Microbiologist, Lederle Laboratories, Pearl River, N. Y. (Antibiotics, virology.)

Weiss, Freeman A. Curator, American Type Culture Collection, 2029 M St., N. W., Washington 6, D. C. (Plant disease bibliography, cultivation and preservation of microorganisms and viruses.)

Weitz, Harold. "C" Division, Camp Detrick, Md. (General pathology.)

Welch, Aaron W. Technical Field Specialist, E. I. du Pont de Nemours & Co., 101 W. 10th St., Wilmington, Del. (Soil fumigants and fungicides.)

Welch, Donald S. Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Forest pathology.)

Wellhausen, Edwin J. Local Director, Rockefeller Foundation Agricultural Program, Londres 45, Mexico, D. F., Mexico. (Plant genetics, corn breeding.)

Wellman, Richard H. Head, Biological Research Department, Carbide and Carbon Chemicals Company, 1086 N. Broadway, Yonkers 3, N. Y. (Agricultural chemicals.)

Wells, Homer D. Agent, Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Georgia Coastal Plain Experiment Station, Tifton, Ga. (Diseases of forage crops.)

Wells, J. C. Assistant Extension Plant Pathologist, North Carolina State College, Box 5397, Raleigh, N. C.

Welsh, Maurice F. Plant Pathologist, Dominion Laboratory of Plant Pathology, Science Service, Summerland, British Columbia, Canada. (Diseases of tree fruits, viruses and virus diseases.)

Wernham, Clifford C. Associate Professor of Plant Pathology, Department of Botany, The Pennsylvania State College, State College, Pennsylvania. (Disease resistance, diseases of corn.)

West, Erdman. (Life) Mycologist and Botanist, Department of Plant Pathology, Florida Agricultural Experiment Station, Gainesville, Fla., Professor of Botany, Department of Botany, College of Agriculture, University of Florida, Gainesville, Fla. (Camellia diseases, Myxomycetes, Florida plants.)

West, Harry. 56 S. Vernon St., Middleport, N. Y.

Westcott, Cynthia. The Plant Doctor, 96 Essex Ave., Glen Ridge, N. J. (Diseases of ornamentals, fungicides for home gardens.)

Wester, Horace V. Plant Pathologist, National Capital Parks, Department of Interior, Washington, D. C. (Ornamentals.)

Weston, William H. (Life) Biological Laboratories D 482, Harvard University, Cambridge, Mass.

Wharton, David C. Graduate Student, Department of Botany, The Pennsylvania State College, State College, Pa. (Viruses, chemotherapy.)

Wheeler, Harry E. Assistant Professor of Botany and Plant Pathology, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge 3, La. (Fungus genetics and physiology, radiobiology.)

Wheeler, Willis H. Plant Pathologist, Division of Plant Quarantines, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington 25, D. C. (Plant quarantine mycology and plant pathology.)

Whipp, Arthur A. Research Entomologist, California Spray Chemical Corporation, 30 West Forest Circle, Orlando, Fla.

Whitaker, Thomas W. Senior Geneticist, Division of Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, P. O. Box 150, La Jolla, Calif. (Plant breeding for disease resistance in lettuce and cantaloupes.)

White, (Mrs.) Irene. Vanderbilt University, Nashville, Tenn.

White, N. H. Faculty of Agriculture, University of Sydney, New South Wales, Australia.

White, Richard P. (Life) Executive Secretary, American Association of Nurserymen, Inc., 635 Southern Building, Washington 5, D. C.

Whitehead, Marvin D. Assistant Professor of Plant Pathology, Department of Plant Physiology and Plant Pathology, Texas Agricultural and Mechanical College, College Station, Tex. (Field crop diseases, seed-borne diseases, mycology.)

Whitney, Will A. (Life) Overnight Editor, News Division WGN, Inc., Tribune Tower, Chicago 11, Ill.

Wiehe, P. O. Chamber of Agriculture, Port Louis, Mauritius, Africa.

Wieman, J. S. 372 State Office Building, Portland 5, Oreg.

Wilcox, Marguerite S. Assistant Mycologist, Division of Handling, Transportation and Storage, Bureau of Plant Industry, Beltsville, Md. (Small fruit diseases, vitamin assay.)

Wilcoxon, Frank. Statistician, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. (Statistics.)

Wiles, Alfred B. Plant Pathologist, Department of Plant Pathology and Physiology, Mississippi Agricultural Experiment Station and United States Department of Agriculture, State College, Mississippi. (Cotton diseases, disease resistance, seed protectants.)

Wilhelm, Stephen. Assistant Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Verticillium diesase, root infecting fungi, small fruit.)

Wilkinson, Robert E. Associate Professor of Plant Pathology, Department of Plant Pathology, Cornell University, Ithaca, N. Y. (Vegetable diseases, virology.)

Wilks, Jack M. Graduate Student, Botany Department, Oregon State College, Corvallis, Ore. (Virology, stone fruits.)

Williams, Albert S. Graduate Student, Department of Plant Pathology, North Carolina State College, Raleigh, N. C. (Virology.)

Williams, Edwin B. Graduate Assistant, Department of Botany and Plant Pathology, Agricultural Experiment Station, Purdue University, Lafayette, Ind.

Williams, Harold E. 2609 Tyler, Corvallis, Ore.

Williams, L. E. 89 W. Lane Ave., Columbus, Ohio.

Williamson, Charles E. Assistant Professor of Plant Pathology, Cornell University, Ornamentals Research Laboratory, Farmingdale, N. Y. (Diseases of ornamentals, soil fumigants.)

Willison, Robert S. Associate Plant Pathologist, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario, Canada. (Stone fruit viruses and diseases.)

Wilson, Alasdair R. (Life) Scientific Officer-in-Charge, Agricultural Research Council Potato Storage Investigation Team, % University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire, England. (Diseases and storage of potatoes.)

Wilson, Coyt T. Assistant Dean and Director, School of Agriculture and Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn, Alabama. (Fungus physiology, fungicides.)

Wilson, Edward E. Professor of Plant Pathology and Plant Pathologist, Department of Plant Pathology, University of California, Davis, Calif. (Fruit diseases, fungicides.)

Wilson, Elmer L. Plant Quarantine Inspector, Division of Plant Quarantines, Bureau of Entomology and Plant Quarantines, United States Department of Agriculture, Plant Quarantine Station, 3 Anza Dr., Nogales, Ariz. (Plant diseases, entomology.)

Wilson, J. Dean. Professor of Plant Pathology, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station and Ohio State University, Wooster, Ohio. (Vegetable diseases, fungicides, spray adjuvants, and application machinery.)

Wilson, N. S. 3761 Bandini Ave., Riverside, Calif.

Wilson, Orville T. Department of Botany, University of Cincinnati, Cincinnati 21, Ohio.

Wilson, V. E. Pathologist, United States Department of Agriculture, Box 1100, Twin Falls, Idaho.

Wilson, William E. Assistant Professor of Botany, Department of Botany, Miami University, Oxford, Ohio. (Teaching, fungus physiology.)

Wingard, Samuel A. Professor of Plant Pathology and Head of Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Va. (Fungus diseases, breeding for disease resistance.)

Winstead, Nash N. Graduate Student, Department of Plant Pathology, University of Wisconsin, Madison 6, Wis.

Winston, John R. Senior Horticulturist, United States Department of Agriculture, Orlando, Fla. (Post-harvest pathology, parasitic-decay control.)

Winter, Floyd L. Director, Breeding and Research, Associated Seed Growers, Inc., P. O. Box 406, New Haven 2, Conn. (Vegetable improvement, plant physiology, disease resistance.)

Winter, H. Frank. Associate Professor of Plant Pathology, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio. (Fruit diseases, fungicides.)

Wischhusen, John F. Consultant on Trace Elements, Inorganic Bioelements, Inc., 15037 Shore Acres Dr., Cleveland 10, Ohio.

Wismer, Chester A. Associate Plant Pathologist, Experiment Station, Hawaiian Sugar Planters' Association, Honolulu 14, Hawaii. (Sugar cane diseases.)

Wolcyrz, Sylvia. Research Assistant, Department of Botany, University of Illinois, Urbana, Ill. (Virology and mycology.)

Wolf, Frederick A. (Charter, Life) Professor of Botany, Duke University, Durham, N. C. (Fungi.)

Wolf, Frederick T. Associate Professor of Biology, Department of Biology, Vanderbilt University, Nashville 5, Tenn. (Physiology of fungi.)

Wolfe, Homer R. Plant Pathologist, Washington State Department of Agriculture, Tree Fruit Experiment Station, Wenatchee, Wash. (Insect vectors of stone fruit virus diseases.)

Wollman, E. S. H. Botany Department, Ohio State University, Columbus 10, Ohio.

Wood, Jessie I. Associate Pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils and Agricultural Engineering, Plant Industry Station, Beltsville, Md. (Plant disease survey.)

Wood, Leon S. Graduate Student, Division of Plant Pathology, University of Minnesota, University Farm, St. Paul, Minn. (Cereal diseases.)

Wood, William B. In Charge, District of Columbia Inspection, United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, Washington, D. C.

Woodbury, Elton N. Chief Entomologist, Hercules Powder Company, Wilmington, Del. (Fungicides.)

Woods, Mark. 4003 Quintana St., Hyattsville, Md.

Woudhuysen, Henry L. Research Chemist, H. L. Woudhuysen and Associates, 17 Battery Pl., New York 4, N. Y. (Fungicides.)

Wright, Charles M. Associate Professor of Plant Pathology, Washington State College, Pullman, Wash. (Teaching and virology.)

Wright, Ernest. (Life) Pathologist, Division of Forest Pathology, Bureau Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, P. O. Box 4137, Portland, Ore. (Tree diseases, forest nursery diseases, soil microbiology, decay of forest products.)

Wright, L. Kenneth. In Charge Administrative Services, Bureau Entomology and Plant Quarantine, United States Department of Agriculture, Washington 25, D. C. (Cereal diseases.)

Wright, Theodore R. Associate Pathologist, United States Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, P. O. Box 99, Wenatchee, Wash. (Diseases occurring in handling, transportation, and storage of fruits.)

Wyllie, Thomas D. Graduate Student, Department of Plant Pathology, University of Minnesota, University Farm, St. Paul, Minn. (Industrial mycology, mycology, viruses.)

Wysong, Noel B. Chief Forester of the Forest Preserve District of Cook County, 536 N. Harlem Ave., River Forest, Ill. (Forest and shade tree diseases.)

Yale, John Wesley, Jr. Graduate Research Assistant, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Vegetable diseases, virology.)

Yarwood, C. E. Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley 4, Calif. (Fungicides, obligate parasites, virology.)

Yerkes, William D., Jr. Assistant Plant Pathologist, The Rockefeller Foundation, Calle Londres 45, Mexico 6, D. F., Mexico. (Vegetable diseases, mycology.)

Yoder, Donald M. Senior Fellow, Plant Pathology, Boyce Thompson Institute for Plant Research, Inc., and Biological Research Division, Carbide and Carbon Chemicals Company, 1086 N. Broadway, Yonkers 3, N. Y. (Mechanism of fungicidal action, fungicide evaluation.)

Yost, Henry E. Golden Nematode Control, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Box 104, Hicksville, Long Island, N. Y.

Young, Harry C. Associate Chairman, Department of Botany and Plant Pathology, and Professor of Plant Pathology, Ohio Agricultural Experiment Station and Ohio State University, Wooster, Ohio. (Fungicides, fungus physiology.)

Young, Harry C., Jr. Associate Professor of Plant Pathology, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla. (Cereal diseases.)

Young, Paul A. (Life) Plant Pathologist in Charge, Tomato Disease Laboratory, Texas Agricultural Experiment Station, Jacksonville, Tex. (Disease and genetics of tomatoes.)

Young, Roy A. Associate Professor of Plant Pathology and Associate Plant Pathologist, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore. (Diseases of ornamental plants and potatoes, fungicides.)

Young, V. H. Professor of Plant Pathology and Head of Department of Plant Pathology, University of Arkansas, Plant Pathologist, Arkansas Agricultural Experiment Station, Plant Pathologist, Arkansas State Plant Board, 226 Agricultural Building, University of Arkansas, Fayetteville, Ark. (Cotton diseases, fungus physiology, fungal enzymes.)

Young, William J. Graduate Student, Department of Bot-

any and Plant Pathology, Michigan State College, East Lansing, Mich. (Fruit diseases.)

Younkin, Stuart G. Assistant to Director of Research, Research and Development Department, Campbell Soup Company, Camden 1, N. J. (Vegetable diseases.)

Yount, William L. Plant Pathologist, Pennsylvania Department of Agriculture, Harrisburg, Pa. (Diseases of nursery and certified seed crops, identification and eradication of plant diseases.)

Yusef, Hasan M. Faculty of Science, Farouk I University, Alexandria, Egypt.

Zabel, Robert A. Assistant Professor of Forest Pathology, Department of Botany and Forest Pathology, College of Forestry, State University of New York, Syracuse, N. Y. (Forest pathology, wood deterioration, fungicides, forestry.)

Zaitlin, Milton. Graduate Student, University of California at Los Angeles, Los Angeles 24, Calif. (Virology.)

Zambettakis, Charalambos E. Phytopathologist-Agronome, Attaché de Recherches au Centre National de le Rechereche Scientifique de France. Maison Internationale, 19 Bd. Jourdan, Paris 14e, France. (Plant diseases in warm countries.)

Zaumeyer, W. J. Principal Pathologist, United States Department of Agriculture, Plant Industry Station, Beltsville, Md. (Bean and pea diseases.)

Zentmyer, George A. Associate Plant Pathologist, Department of Plant Pathology, University of California, Riverside, Calif. (Fungicides, avocado diseases, fungus physiology, chemotherapy.)

Zoril, Joseph G. Plant Pathologist, Research Division, Chicago Carnation Growers' Association, 1670 Orchard St., Des Plaines, Ill. (Mycology, antibiotics, physiology.)

Zscheile, Frederick Paul. Professor of Agronomy and Biochemist in the Experiment Station, University of California, Davis, Calif. (Nature of disease resistance in plants, fungus physiology, plant biochemistry.)

Zuck, Robert K. Associate Professor and Head, Department of Botany, Drew University, Madison, N. J. (Breeding for disease resistance, fiber deterioration, phylogeny of fungi.)

Zuckerman, Bert M. Assistant Plant Pathologist, Illinois State Natural History Survey, 385 Natural Resources Building, Urbana, Ill. (Tree diseases.)

Zuckerman, Herbert G. Farmer, Peat Soils in California, Mineral Soils in Utah and Oregon, 410 Bank of America Building, Berkeley 4, Calif. (Potatoes, onions, grains, fodder and forage plants.)

SUPPLEMENTARY LIST

Additional members not included above nor in Geographical Index

Adams, Robert E. Graduate Student, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Allard, Robert W. Associate Professor and Associate Agronomist, University of California, Davis, Calif.

Berkeley, Edmund. Assistant Professor of Biology, University of the South, Sewanee, Tenn.

Brakke, Myron K. Department of Botany, University of Illinois, Urbana, Ill. (Virology.)

Chessin, Meyer, Assistant Professor, Botany Department, Montana State University, Missoula, Mont.

Culver, Andrew J., Jr. Plant Pathologist in Charge, United States Department of Agriculture, Production and Marketing Administration, Insecticide Division, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Ore.

Echandi, Eddie. Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Fernow, Karl H. Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Garcia, Adrien. Chef, Département Agrochimie, Societe Somechange S. A., Service Prod. Chimiques, 34 Rue Polignac, Alger, Algeria.

MacLachlan, D. S. Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Ohle, Lester C. Field Representative, Calkins Manufacturing Company, Spokane, Wash.

Olien, Charles Robert. Research Assistant, Department of Plant Pathology, University of Minnesota, St. Paul, Minn.

Pappo, Shimon. Department of Plant Pathology, University of California, Davis, Calif.

Patino, Bernardo. Box 560, San Salvador, Salvador.

Peleg (formerly Perlberger), Jacob. Plant Pathologist, Head, Division of Plant Protection, Ministry of Agriculture, P. O. B. 8393, Tel-Aviv, Jaffa, Israel.

Pfeifer, Robert P. Professor of Agronomy, Department of Agronomy, University of Wyoming, Laramie, Wyoming. (Small grains breeding, wheat, oats, and barley.)

Speroni, Horacio Augustin. Dirección General, Investigaciones Agricolas, Ministerio de Agricultura y Ganadería, Virrey Melo 2472, Buenos Aires, Argentina. (Citrus diseases.)

Wildman, Sam. Associate Professor, Botany Department, University of California, Los Angeles 24, Calif.

Wintersteen, Hugh. Graduate Assistant, Department of Botany, Iowa State College, Ames, Iowa.

Wooley, Paul H. Graduate Research Assistant, Department of Botany and Plant Pathology, Oregon Agricultural Experiment Station, Corvallis, Ore.

Ziver, Abraham. Agronomist, Departamento de Investigaciones Agricolas, Santiago, Chile.

GEOGRAPHICAL INDEX

UNITED STATES, TERRITORIES AND POSSESSIONS

Alabama

Henderson, Lavaniel L.
Self, Raymond L.
Timonin, Michael I.
Alabama Polytechnic Institute, Auburn
Diener, Urban L.
Garren, Kenneth H.
Greenleaf, Walter H.
Lyle, James A.
Seal, J. L.
Smith, Albert L.
Wilson, Coyt T.

Arizona

Booth, J. A.
Brisley, Harold R.
Evans, Stuart
Foster, Robert E., II
Gibson, Frederick
Marlatt, Robert B.
McCormick, W. H.
Reynolds, Harold W.
Shields, Ivan J.
Simonsen, Maryhelen
Wilson, Elmer L.
University of Arizona, Tucson
Allen, Ross M.
Brown, James Greenlief
Dill, Harold A.
Keener, Paul D.

Arkansas

Millar, Paul H.
University of Arkansas, Fayetteville
Cralley, E. M.
Fulton, Joseph P.
Orsenigo, Mario
Rosen, H. R.
Slack, Derald A.
Smith, Harlan E.
Young, V. H.

California

Anderson, F. W.
Ballard, William S.
Bardin, Roy Edwards
Bobrov, Ruth Ann
Bohn, Guy Weston
Bowers, James Basil
Carsner, Eubanks
Carter, R. D.
Damann, J. B.
Davis, Donald W.
de Ong, E. R.
Dimitman, Jerome E.
Dufrenoy, Jean
Dundas, Bjarne
Fairchild, S. J.
Ferris, Curtis A.
Fitzpatrick, Hugh F.
Fong, Richard B.
Gardner, Leo R.
Graves, C. E.
Hampton, Jack E.
Hanson, R. Gordon
Harrison, George J.
Harvey, John M.
Hatfield, W. C.
Hill, John P.
Holloman, Arthur, Jr.
Hudson, Roy
Humphrey, Clarence J.
Humphrey, Harry B.
Hunt, N. Rex
Kortsen, Robert A.
Lachmund, Harry G.
Laflin, Benjamin T., Jr.
Littooy, E. H.
Locke, Warren F.
MacMillan, Howard G.
McClellan, W. D.
McFarlane, John S.
Meinecke, Emilio P.
Meuli, Lloyd J.
Milbrath, David G.
Millerburg, Claus J. W.
Munn, Mancel T.
Nesbitt, Robert B., Jr.
Nickel, J. L.
Nixon, Walter H.
Panasenko, Vasil T.
Porter, Donald R.
Pryor, Dean E.
Rudolph, Bert A.
Saunders, W. B.
Shafer, W. B.
Steinen, Otto P.
Thomas, Harold E.
Thornton, Raymond L.
Wagener, Willis W.
Warner, Robert M.
Weathers, L. G.
Whitaker, Thomas W.
Wilson, N. S.
Utech, Nedra M.
Zuckerman, Herbert G.
California Department of Agriculture, Riverside
French, Alex M.
McClain, R. L.
Rosenberg, Dan Y.
California Department of Agriculture, Sacramento
Altstatt, George E.
Lee, Bertrand W.
Schlocker, Archie
Stout, Gilbert L.
Wagnon, H. Keith
California Packing Corporation, San Leandro
Caldis, Panos D.
Gilmer, Ralph A.
Lawyer, Lewis O.
Virgin, Walter J.
Shell Agricultural Laboratory, Modesto
Kreutzer, William A.
Monroe, Carl M.
Rader, William E.
Turner, Mason M.
United States Department of Agriculture, Riverside
Bennett, Carlyle W.
Cochran, L. C.
Giddings, Nahum James
Lackey, Charles F.
University of California, Berkeley
Alcorn, Stanley M.
Allen, Merlin W.
Ark, Peter A.
Baker, R. Ralph
Barrett, James T.
Bonar, Lee
Frazier, Norman W.
Freitag, Julius H.
Gardner, Max W.
Gold, Herbert
Hansen, H. N.
Harvey, Harry T.
Jensen, Dilworth D.
Kennedy, W. H.
Mattei, John A.
Nelson, Paul E.
Nichols, Carl W.
Nour-Eldin, Farid
Raabe, Robert D.
Raski, Dewey J.
Rawlins, Thomas E.
Santilli, Vincent
Schlegel, David E.
Schneider, Irving R.
Scott, C. Emlen
Simons, John N.
Snyder, William C.
Stanley, Wendell M.
Steere, Russell L.
Sylvester, Edward S.
Takahashi, William N.
Tammen, James
Thomas, Harvey Earl
Tompkins, C. M.
Wilhelm, Stephen
Yarwood, C. E.
University of California, Davis
Abeloe, Barbara B.
Briggs, Fred N.
Couch, Houston B.
Davis, Glen N.
Delp, Charles J.
Edgington, Lloyd V.
English, W. Harley
Grogan, Raymond G.
Henderson, William J.
Hewitt, William B.
Houston, Byron R.
Kendrick, James B.
Leach, Lysle D.
Nelson, Klayton E.
Nyland, George
Ogawa, Joseph M.
Oswald, John W.
Pine, Thomas S.
Purdy, L. H., Jr.
Schaller, Charles W.
Smith, Paul G.
Sommer, N. F.
Starr, Mortimer P.
Wilson, Edward E.
Zscheile, Frederick Paul
University of California, Los Angeles
Baker, Kenneth F.
Bald, John G.
Cohen, Morris
Davis, Lily H.
Durbin, Richard D.
Ferguson, John
Markley, Brian B.
Miller, Pierre A.
Munnecke, Donald E.
Plunkett, Orda A.
Siegel, Albert
Zaitlin, Milton

University of California, Riverside
Baines, Richard C.
Calavan, E. Clair
Darley, Ellis F.
Desjardins, Paul R.
Dickson, Robert C.
Erwin, Donald C.
Flock, Robert A.
Kendrick, James B.
Middleton, John T.
Schneider, Henry
Smith, Clayton O.
Treshow, Michael
Troxel, A. W.
Wallace, James M.
Zentmyer, George A.

Colorado

Feichtmeir, Edmund F.
Griffith, R. B.
Pinckard, J. A.
Sisson, Max A.
Swingle, John W.
Colorado Agricultural and Mechanical College, Fort Collins
Davidson, Ross W.
Dickens, Lester E.
Faulkner, Lin R.
Foeppel, William G.
Gaskill, John O.
Gerhold, Norman R.
Lane, George H.
Porter, R. Howard
Seliskar, Carl E.
Thomas, Walter D., Jr.

Connecticut

Anderson, Paul J.
Caroselli, Nestor E.
Cochrane, Vincent W.
Desrosiers, Russell
Feldman, Albert W.
Gordon, Fred M.
Graves, Arthur Harmount
Karsten, Kenneth S.
Marshall, Rush P.
Nolan, Kenneth G.
Shepherd, H. R.
Stark, Frank L.
Tate, H. Douglas
Torrey, G. Safford
Associated Seed Growers, Inc., New Haven
Nutile, Gabriel E.
Tidd, Joseph S.
Winter, Floyd L.
Connecticut Agricultural Experiment Station, New Haven
Davis, David
Dimond, Albert E.
Horsfall, James G.
Lownsbery, Benjamin F.
Nienstaedt, Hans
Rich, Saul
Stoddard, Ernest M.
Taylor, Gordon S.
Waggoner, Paul E.
United States Department of Agriculture, New Haven
Hahn, Glenn G.
Mook, Paul V.
Spaulding, Perley
Waterman, Alma M.
Yale University, New Haven
Boyce, John S.
Grossbard, Erna
Pollard, Ernest C.

Delaware

Adams, James F.
Owen, Frank C.
E. I. du Pont de Nemours & Company, Inc., Wilmington
Bucha, Harry C.
Evans, Joseph A.
Krause, Robert L.
Krister, Charles J.
McGahen, J. W.
Miles, G. F.
Richards, B. Lorin, Jr.
Ryker, Truman C.
Stoddard, David L.
Tisdale, W. H.
Welch, Aaron W.
Hercules Powder Company, Wilmington
Palmer, Hugh C.
Stessel, George J.
Woodbury, Elton N.
University of Delaware, Newark
Comegys, Wallace R.
Cox, R. S.
Crittenden, H. William
Heuberger, John W.
Hyre, Russell A.
Manns, Thomas F.
Porter, Frank M.

District of Columbia

Bamberg, Rayburn H.
Brown, Nellie A.
Charles, Vera K.
Edson, Howard A.
Ezekiel, Walter Naphtali
Fulton, Harry R.
Hitchner, L. S.
Hough, L. Fredric
Mahoney, Charles H.
McCulloch, Lucia
Schlatter, F. P.
Stewart, Dewey
Van Meter, Mary G.
Vestal, Edgar F.
Waldee, E. L.
Weiss, Freeman A.
Wester, Horace V.
White, Richard P.
United States Department of Agriculture
Dean, Horace S.
Fracker, Stanley B.
Fromme, F. D.
Haskell, Royal J.
Kevorkian, Arthur G.
LeClerg, E. L.
Lefebvre, C. L.
Liming, O. Neal
Reinking, Otto A.
Sasscer, E. R.
Walker, Earnest A.
Wheeler, Willis H.
Wood, William B.
Wright, L. Kenneth

Florida

Bourne, Benjamin Arthur
Brooks, A. Nelson
Brooks, Charles
Christie, James R.
Conover, Robert A.
Crall, James M.
Cunniff, Hilda Spink
Darby, John F.
Eddins, Auther H.
Hobbs, Gordon A.
Hogan, William D.
Jacoway, Thomas H.
Kincaid, Randall R.
Large, John R.
Magie, Robert O.
Maxwell, Lewis S.
Meyers, Samuel P.
Moore, William D.
Newberger, Edward F.
Olive, Edgar W.
Peterson, Paul D.
Rands, Robert D.
Reddick, Donald
Rhoads, Arthur S.
Roberts, John W.
Ruehle, George D.
Slade, Martin A., Jr.
Swank, George, Jr.
Tomasello, R. P.
Townsend, George R.
Voorhees, R. K.
Walter, James M.
Whipp, Arthur A.
University of Florida, Gainesville
Anderson, Chris W.
Decker, Phares
Earhart, Robert W.
Gratz, Levi Otto
Miller, Howard M.
Muller, A. S.
Owen, John H.
Senn, Pettus H.
Tisdale, William B.
Weber, George F.
West, Erdman
Citrus Experiment Station, Lake Alfred
Cohen, Mortimer
DuCharme, Ernest P.
Fisher, Francine E.
Knorr, L. Carl
Pratt, Robert M.
Suit, R. F.
Everglades Experiment Station, Belle Glade
Pate, James B.
Stevenson, Frank V.
Stoner, Warren N.
Summers, Thomas E.
United States Department of Agriculture, Orlando
Childs, J. F. L.
Grant, Theodore J.
Winston, John R.

Georgia

Alden, John C.
Bruer, Howard L.
Cole, J. R.
Foster, A. Alfred
KenKnight, Glenn
Nikitin, Alexander A.
Persons, Thomas D.
Shepard, Calvin E.
Taylor, Jack
Ukkelberg, Harry G.
University of Georgia, Athens
Campbell, William A.
Jackson, Lyle W. R.
Miller, Julian H.
Thompson, George E.
Georgia Agricultural Experiment Station, Experiment

Boyle, Lytton W.
Higgins, B. B.
Luttrell, E. S.
Georgia Coastal Plain Experiment Station, Tifton
Borders, Huey I.
Gill, Denzell L.
Machmer, John H.
Rankin, Harvey W.
Wells, Homer Douglas

Idaho

Anderson, Melvin E.
Brown, Clarke
Dean, Leslie L.
Finley, Arthur M.
Murphy, Albert M.
Pierce, Walter H.
Wilson, V. E.
University of Idaho, Moscow
Gauger, Wendell L.
Helton, A. W.
Hubert, Ernest E.
Hungerford, Charles W.
Raeder, John Milford
Slipp, Albert W.
Watson, Roscoe D.
Branch Experiment Station, Aberdeen
Dallimore, Clarence Eugene
Guthrie, James W.
McLean, John Grim

Illinois

Abramitis, Walter W.
Andrews, Howard J.
Brown, (Mrs.) Pembroke H.
Bussart, J. Everett
Deems, R. E.
Fiore, Charles
Geise, Fred W.
Hesseltine, C. W.
Heiberg, Barbara C.
Holbert J. R.
Johnson, Nels J.
Kitchen, E. M.
Link, George K. K.
Mowry, James B.
Nordlinger, Ernest W.
Piersma, Sidney F.
Piesbergen, N.
Ramsey, Glen B.
Sikora, Berwin R.
Smith, Marion A.
Spencer, Edwin R.
Whitney, Will A.
Wysong, Noel B.
Zoril, Joseph G.
Illinois State Natural History Survey, Urbana
Boewe, G. H.
Carter, James Cedric
Curl, Elroy A.
Forsberg, Junius L.
Himelick, Eugene B.
Hoffman, Paul F.
Schein, Richard D.
Schenck, Norman Carl
Tehon, Leo R.
Zuckerman, Bert M.
University of Illinois, Urbana
Anderson, Harry W.
Beraha, Louis
Bever, Wayne M.
Black, L. M.
Chamberlain, Donald W.
Gerdemann, J. W.
Gottlieb, David
Hickman, D. D.
Koehler, Benjamin
Linford, M. B.
Linn, Manson B.
Miller, P. M.
Powell, Dwight
Shanor, Leland
Takeshita, Robert M.
Thornberry, H. H.
Wolcyrz, Sylvia

Indiana

Jump, John A.
Smucker, Silas J.
Starcs, Karlis
Thanos, Andrew
Ulman, Paul T.
Purdue University, Lafayette
Athow, Kirk L.
Caldwell, Ralph M.
Cummins, George B.
Gries, George A.
Lewis, Gwynne D.
McClintock, J. A.
Patterson, Fred L.
Porter, Charles L.
Schafer, John F.
Shay, J. Ralph
Ullstrup, Arnold J.
Williams, Edwin B.

Iowa

Chao, S. John
Jarvis, Karl H.
Kivilaan, Aleksander
Nitz, Carl G.
Sacco, Paul
Vaughan, R. E.
Iowa State College, Ames
Baxter, John W.
Bragonier, Wendell H.
Buchholtz, Walter F.
Dunleavy, John M.
Engelhard, Arthur W.
Gilman, Joseph C.
Hampton, Richard O.
Hobart, Oscar F., Jr.
Hooker, Arthur L.
Hooker, William J.
Huffman, Marion D.
Lewis, Richard M.
McNabb, Harold S., Jr.
Melhus, Irving E.
Murphy, Hickman C.
Pulsifer, Herbert G.
Reddy, Charles S.
Sharp, Eugene L.
Sherf, Arden F.
Simons, Marr D.
Staffeldt, Eugene E.
Tiffany, Lois H.
Timian, Roland G.
Wallin, Jack R.

Kansas

Bellingham, Roscoe C.
Mix, A. J.
Raleigh, Walter P.
Kansas State College, Manhattan
Eisenstark, A.
Elmer, Otto H.
Hansing, Earl D.
Haskett, William C.
Haymaker, H. Henley
Heyne, E. G.
Johnston, Charles O.
King, Claude L.
Melchers, Leo E.
Pady, Stuart M.
Rogerson, Clark T.
Schafer, Lewis A.
Sill, Webster H., Jr.
Slagg, C. M.

Kentucky

Davy, Roger H.
Lair, Eugenie D.
University of Kentucky, Lexington
Chapman, Richard A.
Chitwood, B. G.
Diachun, Stephen
Henson, Lawrence
Johnson, E. M.
Stokes, Granville W.
Troutman, Joseph L.
Valleau, William Dorney

Louisiana

Abbott, Ernest V.
Exner, Beatrice Barbara
Nesom, Rivers Y.
Phillips, Ruth P.
Schexnayder, Charles A.
Shear, Cornelius Lott
Speairs, Richard K., Jr.
Louisiana State University, Baton Rouge
Baxter, Luther W., Jr.
Birchfield, Wray
Chilton, St. John P.
Dykstra, T. P.
Edgerton, Claude W.
Farrar, Luther L.
Flint, L. H.
Horn, Norman L.
Johnson, Leander F.
Luke, Herbert H.
Martin, W. J.
Morton, Donald J.
Neal, David C.
Plakidas, A. G.
Schwegmann, Jack C.
Steib, René J.
Stroube, William H.
Tims, Eugene C.
Webb, Raymon E.
Wheeler, Harry E.

Maine

Hurst, John W., Jr.
Mollicone, Marilyn Noyes
University of Maine, Orono
Bonde, Reiner
Folsom, Donald
Hilborn, Merle T.
Simpson, Geddes W.
Steinmetz, Ferdinand H.

Maryland

Beattie, R. Kent
Cohill, Roger W.
Demaree, Juan B.
Eck, Richard V.
Elliott, Charlotte
Gerberg, Eugene J.

Huffington, Jesse M.
Hunter, Herman A.
Long, William G.
McCubbin, W. A.
Meier, (Mrs.) F. C.
Showalter, Joseph W.
Stoneleigh, Duane
Ter Horst, W. P.
Woods, Mark
Camp Detrick, Frederick
Bromfield, Kenneth R.
Emge, Robert G.
Kahn, Robert P.
Kingsolver, Charles H.
Latterell, Frances Meehan
Miller, William E.
Mitchell, John E.
Peet, Clyde E.
Pon, Dick S.
Schmitt, Chris G.
Smoot, John J.
Taylor, Carlton F.
Tullis, Edgar C.
Weitz, Harold
United States Department of Agriculture, Beltsville
Barker, Henry D.
Baskin, A. David
Bouchereau, Paul E.
Brierley, Philip
Cairns, Eldon J.
Carter, Edward P.
Cash, Edith K.
Cash, Lillian C.
Cheo, Pen Ching
Clayton, Edward E.
Cook, Harold T.
Cooley, J. S.
Coons, George H.
Darrow, George M.
Diehl, William W.
Doolittle, Sears P.
Drechsler, Charles
Dunegan, John C.
Ellis, Agnes M.
Fowler, Marvin E.
Golden, Morgan
Gravatt, Annie R.
Gravatt, G. Flippo
Hartley, Carl
Hutchins, Lee M.
Johnson, Aaron G.
Kreitlow, Kermit W.
Lambert, Edmund B.
Lautz, William H.
Leukel, Robert W.
Lombard, (Mrs.) Frances F.
Lowther, Conley V.
Marcus, Carlton P., Jr.
Marshall, Barton H., Jr.
May, Curtis
McColloch, Lacy P.
McKinney, Harold Hall
Miller, Paul R.
Nance, Nellie W.
O'Brien, Muriel J.
Porte, William S.
Presley, John T.
Robert, Alice L.
Rodenhiser, Herman A.
Schultz, Eugene S.
Sherwin, Helen S.
Smith, Floyd F.
Smith, Wilson L., Jr.
Steiner, G.
Stevenson, F. J.
Stevenson, John A.
Tapke, Victor F.
Taylor, Albert L.
Thomas, Charles A.
Thomas, H. Rex
Watson, Alice J.
Wilcox, Marguerite S.
Wood, Jessie I.
Zaumeyer, W. J.
University of Maryland, College Park
Cox, Carroll E.
Jeffers, Walter F.
Jehle, Robert A.
Kantzes, James G.
Keller, John R.
Marshall, Norton L.
McGrew, John R.
Morgan, Omar D., Jr.
Oteifa, Bakir A.
Rushdi, Medhat H. K.
Weaver, L. O.

Massachusetts

Bartley, Clayton E.
Beauchamp, Julio T.
Bergman, Herbert F.
Boyd, William I.
Faull, Joseph Horace
Martin, G. Hamilton, Jr.
Matz, Julius
Pearson, O. H.
Simmons, Sidney A.
Weston, William H.
University of Massachusetts, Amherst
Banfield, Walter M.
Doran, William L.
Gilgut, Constantine J.
Kightlinger, Clifford V.
Marsden, David H.
McKenzie, Malcolm A.
Osmun, A. Vincent
Waltham Field Station, Waltham
Gasiorkiewicz, Eugene C.
Guba, Emil F.
Rusden, Philip L.

Michigan

Brian, Charles E.
Drew, William B.
Fraser, Willard S.
Gruenhagen, Richard H.
Hardy, John L.
Karlovic, John K.
Povah, Alfred H.
Prielipp, Donald Otto
Michigan State College, East Lansing
Andersen, Axel L.
Andrews, Edward A.
Barnes, Ervin H.
Bessey, Ernest A.
Bockstahler, Harold W.
Cation, Donald
Clum, Floyd M.
Corcos, Alain
deZeeuw, Donald J.
Fulton, Robert H.
Kiesling, Richard L.
Klomparens, William
Lewis, Ralph W.
Lucas, Eugene H.
McClure, Thomas T.
Mooar, Marie
Muncie, J. H.
Myers, James C., Jr.
Nelson, Ray
Ringel, Samuel M.
Strong, Forrest C.
Strong, Miriam C.
Trytten, Grover W.
Vaughn, John R.
Young, William J.
University of Michigan, Ann Arbor
Baxter, Dow Vawter
Mains, Edwin B.
Scott, William W.

Minnesota

Anderson, C. George
Fletcher, Donald G.
Freeman, E. M.
French, David W.
Johnson, Herbert G.
Mader, Erich O.
University of Minnesota, St. Paul
Ali, Syed B.
Anderson, Ralph L.
Aragaki, Minoru
Bissonnette, Howard L.
Broyles, James W.
Burgess, Richard F.
Butler, Edward E.
Campbell, Robert N.
Christensen, J. J.
Cotter, Ralph U.
DeVay, James E.
Dosdall, Louise T.
Drescher, R. F.
Eide, Carl J.
Fridlund, Paul R.
Frosheiser, Fred I.
Haglund, Frances E.
Hart, Helen
Hayden, E. B.
Hildreth, R. C.
Jackson, Elsa L.
Keplinger, Joseph A.
Kernkamp, Milton F.
King, Thomas H.
Levine, Moses N.
Logsdon, Charles E.
Mekila, Armas A.
Mohamed, Hosni A.
Moore, M. B.
Nelson, Richard R.
Olmstead, Donald S.
Paharia, Kishan D.
Papavizas, George C.
Ram, C. S. Venkata
Reilly, Joseph J.
Rose, Raymond C.
Rowell, John B.
Schneider, Charles L.
Schroeder, Harry W.
Singh, Sheodhan
Smith, David H., Jr.
Stakman, E. C.
Stewart, Donald M.
Swaebly, Mary Ann
Thurston, H. David
Tuite, John F.
Turk, Fateh Mohomed
Wood, Leon S.
Wyllie, Thomas D.
United States Department of Agriculture, Minneapolis
Bulger, Raymond O.

Melander, L. W.
Putnam, Henry N.
Smith, Harry L.

Mississippi

Bain, Douglas C.
Dean, Jack L.
Henry, Berch W.
Mississippi State College, State College
Goode, Monroe J.
Graves, Clinton H., Jr.
Hare, Woodrow W.
Ivanoff, Spas S.
Parris, G. K.
Wiles, Alfred B.
Delta Branch Experiment Station, Stoneville
Bowman, Donald H.
Johnson, Howard W.
Kilpatrick, R. A.
Toole, E. Richard

Missouri

Dodge, Carroll W.
Flippin, Reid S.
Graham, Richard W.
Gregory, Charles T.
Knote, Charles E.
Moore, George T.
Shumard, Roland S.
University of Missouri, Columbia
Bretz, T. W.
Lutes, Dallas D.
Michaelson, Merle E.
Millikan, D. F.
Swartwout, Harold G.
Tucker, C. M.

Montana

Afanasiev, Mitrofan M.
Morris, H. Elwood

Nebraska

Felton, M. W.
University of Nebraska, Lincoln
Allington, William B.
Boosalis, Michael G.
Christiansen, Dwight W.
Goss, Robert W.
Jedlinski, Henry
Jones, John P.
Livingston, Jesse E.
Pool, R. J.
Ray, W. Winfield
Schuster, Max L.
Weihing, John L.

Nevada

Smith, Oliver F.

New Hampshire

Kuss, Frederick R.
McLaughlin, G. A.
Rich, Avery E.
Richards, Mathias C.

New Jersey

Ballantyne, Donald L., Jr.
Braun, Armin C.
Dulaney, Eugene L.
Fields, Walter S.
Miller, Joseph A.
Owen, Emily M.
Powers, William J.
Reed, T. Walter
Rex, Edgar G.
Snyder, H. Donald
Tischler, Nathaniel
Utter, L. Gordon
Westcott, Cynthia
Younkin, Stuart G.
Zuck, Robert Karl
Campbell Soup Company, Riverton
Hoadley, Alfred D.
Reynard, George B.
Weber, Paul V. V.
Rutgers University, New Brunswick
Bachelder, Stephen
Bruskin, Syril
Cohoon, D. Fred
Daines, Robert H.
Davis, Benjamin H.
Davis, Spencer, H., Jr.
Goheen, Austin C.
Haenseler, C. M.
Martin, William H.
Varney, Eugene H.
United States Department of Agriculture, Hoboken
Fenner, Lawrence M.
Limber, Donald P.
Roth, Herbert

New Mexico

Hawksworth, Frank G.
Hsi, David C. H.
New Mexico College of Agriculture and Mechanic Arts, State College
Blank, Lester M.
Chilton, John E.
Hunter, Richard E.
Lear, Bert
Leyendecker, Philip J., Jr.
Nakayana, Roy M.

New York

Apostolides, C. A.
Beale, Helen Purdy
Brandwein, Paul F.
Brozdowski, John J., Jr.
Cetas, Robert C.
Clum, Harold H.
Cohen, Sylvan I.
Colley, Reginald H.
Cowan, E. Karl
Cunningham, Howe S.
Dodge, Bernard O.
Dye, H. W.
Emerson, Frank H.
Feder, William A.
Feldmesser, Julius
Florentine, S. J.
Gemmell, Louis G.
Gloyer, Walter O.
Gonzalez, F. Eugene
Gotz, M. B.
Hall, William C.
Harrar, J. George
Harry, John B.
Ho, William T. H.
Horsey, Robert E.
Inman, Forrest G.
Kolk, Laura A.
Konzak, Calvin F.
Livingston, A. M.
MacNair, James D.
Mason, Curtis L.
McKelvey, J. J.
McManus, P. J.
Mehrlich, F. P.
Palmiter, DeForest H.
Parker, E. K.
Pirone, Pascal P.
Poland, George L.
Porter, Richard P.
Rankin, W. H.
Santacroce, Nunzio G.
Schwarze, C. A.
Shear, E. V.
Small, Cyril G.
Visor, Frederick C.
West, Harry
Williamson, Charles E.
Woudhuysen, Henry L.
Yost, Henry E.
Boyce Thompson Institute, Yonkers
Althaus, Ralph E.
Goenaga, A.
McCallan, S. E. A.
McNew, George L.
Miller, Lawrence P.
Norton, Robert J.
Owens, Robert G.
Palm, Elmer Thurman
Porter, Clark A.
Schuldt, Paul H.
Torgeson, D. C.
Vlitos, August John
Weed, Richard M.
Wellman, Richard H.
Yoder, Donald M.
College of Forestry, Syracuse
Hirt, Ray R.
Silverborg, Savel B.
Zabel, Robert A.
Cornell University, Ithaca
Ahmedi, Ahmad A.
Barrus, Mortier F.
Boothroyd, Carl W.
Browning, John Artie
Burkholder, Walter H.
Burrell, Arthur B.
Cappellini, Raymond A.
Ceponis, Michael J.
Chupp, Charles D.
Cocklin, Paul H.
Corbett, Merwin K.
Dickey, Robert S.
Dimock, A. W.
Ferris, John M., III
Fezer, Karl D.
Guterman, Carl E. F.
Hannon, Chancellor I.
Harrison, Martin B.
Hartman, John D.
Holmes, Francis W.
Kent, George C.
Klos, Edward J.
Macdonald, Kenneth H.
Mai, William F.
Massey, Louis M.
Newhall, Allan G.
Pantidou, Maria E.
Parker, Kenneth G.
Peterson, Lester C.
Pieringer, Arthur P.
Roberts, Daniel A.

Rochow, William F.
Rogers, Virginia
Ross, A. Frank
Ross, John P.
Segall, Ralph H.
Sherwood, Robert T.
Silber, Gustave
Sowell, Grover, Jr.
Tyler, L. J.
Welch, Donald S.
Wilkinson, Robert E.
Lederle Laboratories Division, Pearl River
Duggar, Benjamin M.
Porter, John N.
Thomas, Samuel O.
Weindling, Richard
Wilcoxon, Frank
New York State Agricultural Experiment Station, Geneva
Braun, Alvin J.
Gilmer, Robert M.
Hamilton, James M.
Natti, John J.
Schroeder, Wilbur T.
Swenson, Knud G.
Szkolnik, Michael
Rockefeller Institute for Medical Research, New York City
Holmes, Francis O.
Kunkel, Louis O.
Maramorosch, Karl
United States Department of Agriculture, New York City
Burke, Leo James
Friedman, Bernard A.
Friedman, William

North Carolina

Drolsom, Paul N.
Moore, E. Leon
Smith, Cecil G.
Smith, Thomas E.
Warren, John R.
Wolf, Frederick A.
North Carolina State College, Raleigh
Allison, J. Lewis
Apple, J. Lawrence
Clayton, Carlyle N.
Cooper, William E.
Crossan, Donald F.
Debbagh, K. A.
Ellis, Don E.
Fink, Harry C.
Haasis, Frank A.
Hebert, Teddy T.
Jensen, James H.
Kelman, Arthur
Lehman, Samuel G.
Libby, Robert R.
Lucas, George Blanchard
Moseman, John G.
Nielsen, Lowell W.
Nusbaum, Charles J.
Person, Lee H.
Powers, Harry R., Jr.
Sasser, J. N.
Wells, J. C.
Williams, Albert S.
United States Department of Agriculture, Asheville
Boyce, John S., Jr.
Hepting, George H.
Roth, Elmer R.

North Dakota

North Dakota Agricultural College, Fargo
Brentzel, William Edward
Flor, Harold H.
Hoyman, William G.
Schwinghamer, Erwin A.

Ohio

Atwood, Harry
Darker, Grant D.
Davis, V. H.
Eckess, Edgar
Lancaster, Francis Rhodes
Olson, Conrad J.
Porter, Harold L.
Prince, Alton E.
Schoeneweiss, D. F.
Slutz, Russell C.
Standen, John H.
Swingle, Roger U.
Tilford, Paul E.
Vermillion, M. T.
Williams, L. E.
Wilson, Orville T.
Wilson, William E.
Wischhusen, John F.
Battelle Memorial Institute, Columbus
Baldwin, Maynard M.
Chester, K. Starr
Davidson, Richard S.
Kazmaier, Harold E.
McLaughlin, J. Harvey
Munger, George Donald
Ohio Agricultural Experiment Station, Wooster
Alexander, Leonard J.
Bart, George J.
Kommedahl, Thor
Runnels, Harmon A.
Schmitthenner, August F.
Spilker, Oren W.
Thomas, Roy C.
Wilson, J. Dean
Winter, H. Frank
Young, Harry C., Sr.
Ohio State University, Columbus
Allison, Clyde C.
Allison, Patricia
Ellett, Clayton Wayne
Farabee, Gary J.
Foster, Virginia
Herr, Leonard J.
Higgins, Daniel J.
Janson, Blair F.
Meyer, Bernard S.
Panzer, James D.
Reigelman, Gordon L.
Sims, Asa C., Jr.
Smith, Glenn E.
Snowden, Parker
Snyder, K. C.
Stall, Robert E.
Stover, W. C.
Suhovecky, Albert J.
Thayer, Paul L.
Walker, Jerry T.
Wollman, E. S. H.

Oklahoma

Oklahoma Agricultural and Mechanical College, Stillwater
Brinkerhoff, Lloyd A.
Converse, Richard H.
Epperly, Jean R.
Hocker, Reginald O.
Morrison, Lou S.
Roberts, Bill J.
Struble, F. Ben.
Thomas, John E.
Wadsworth, Dallas F.
Young, Harry C., Jr.

Oregon

Barss, Howard P.
Childs, Leroy
Evans, Arlyn W.
Kienholz, Jess R.
Miller, Roy E.
Milliman, Loren H.
Tanner, Carl R.
Wieman, J. S.
Williams, Harold E.
Wright, Ernest
Oregon State College, Corvallis
Barnes, George L.
Corden, Malcolm E.
Dana, Bliss F.
Dietz, S. M.
Dobie, Norman D.
Hardison, John R.
Hoerner, Godfrey R.
Horner, Chester E.
Jensen, Harold J.
Jones, Leo E.
McWhorter, Frank P.
Milbrath, John A.
Miller, Paul W.
Nielson, Mervin W.
O'Reilly, Henry James
Owens, Charles E.
Roth, Lewis F.
Snow, Gordon F.
Vaughan, Edward K.
Wilks, Jack M.
Yale, John Wesley, Jr.
Young, Roy A.

Pennsylvania

Bejuki, Walter M.
Boeshore, Irwin P.
Brandes, Gordon A.
Guy, H. G.
Hubbell, Dean S.
Jarnagin, W. M.
Johnson, T. C.
Lewis, Fred H.
McConnell, Louis J.
Miller, Harold J.
Poulos, Panos L.
Reed, George M.
Rettew, G. Raymond
Statler, Maynard W.
Weaver, Ernest P.
Yount, William L.
Pennsylvania State College, State College
Bauer, Allen H.
Beach, Walter S.
Boyle, John S.
Braverman, Samuel W.
Burke, O. D.
Chandler, Webster A.
Fergus, Charles L.
Foley, Dean C.
Graham, Joseph H.
Held, Victor M.
Honey, Edwin E.
Kern, Frank D.
Kirby, Robert S.

Kneebone, Leon R.
Merek, Edward L.
Mills, Wilford R.
Nichols, Lester P.
Thurston, Henry W., Jr.
Wernham, Clifford C.
Wharton, David C.
Rohm and Haas Company, Bristol
Davies, Fred R.
Froehlich, John P.
Keil, Harry L.
Martin, Helen
University of Pittsburgh, Pittsburgh
Gainor, Charles
Price, W. C.
Trkula, David

Rhode Island

Hood, John J.
Lepore, A.
Snell, Walter H.
University of Rhode Island, Kingston
Howard, Frank L.
Shurtleff, Malcolm C., Jr.
Tarjan, Armen C.

South Carolina

Aycock, Robert
Barnes, William Carroll
Barre, Henry W.
Epps, William M.
Graham, Thomas W.
Hadden, S. J.
Holdeman, Quintin L.
Rogers, C. Hoyt
Clemson Agricultural College, Clemson
Armstrong, George M.
Arndt, Charles H.
Foster, H. H.
Petersen, Donald H.
Poole, Robert Franklin

South Dakota

South Dakota State College, Brookings
Cook, Allyn A.
Nagel, Clatus M.
Semeniuk, George

Tennessee

Epps, James M.
Heggestad, Howard E.
Latham, Dennis H.
Ritchie, Calvin L.
Skoog, Harold A.
White, (Mrs.) Irene
Wolf, Frederick T.
University of Tennessee, Knoxville
Andes, James O.
Felix, Earl L.
Hesler, L. R.
Reed, Howard E.
Sherbakoff, C. D.
Stevens, Russell B.

Texas

Atkins, John G., Jr.
Bird, Luther S.
Burk, Lawrence G.
Harrison, Arthur L.
Hildebrand, Earl M.
Hudgins, Harry R.
Johnson, Howard B.
Lyerly, Paul J.
Lyle, Eldon W.
Poole, D. Donald
Smith, Donald J.
Smith, Edward
Todd, Edwin H.
Young, Paul A.
Texas Agricultural and Mechanical College, College Station
Calvert, O. H.
Das, G. N.
Futrell, Maurice C.
Johnson, Samuel P.
McFadden, E. S.
Menon, Sankara K.
Norton, Don C.
Pilgrim, A. J.
Rosberg, David W.
Watkins, G. M.
Whitehead, Marvin D.
Texas Agricultural Experiment Substation, Weslaco
Godfrey, G. H.
Olson, Edward O.
Sleeth, Bailey

Utah

Dugway Proving Ground, Tooele
Gardner, Wayne S.
Goto, Shosuke
Harvey, James V.
Hotson, Hugh H.
Tervet, Ian W.
Utah State Agricultural College, Logan
Ames, Ralph W.
Cannon, Orson S.
Cochran, George W.
Mielke, James L.
Richards, B. L.
Wadley, Bryce N.

Vermont

Sproston, Thomas, Jr.

Virginia

Bagdon, Vincent J.
Diller, Jesse D.
French, G. Talbot
Groves, Ancell B.
Hanley, Joseph F.
Jenkins, Wilbert A.
Miller, Lawrence I.
Mullin, R. S.
Nugent, Thomas J.
Wallace, Errett
Watson, William M.
Virginia Polytechnic Institute, Blacksburg
Fenne, S. B.
Henderson, Robert G.
Roane, Curtis W.
Wingard, Samuel A.

Washington

Chapman, Clarence M.
Gibson, Kenneth E.
Harrison, Charles H.
Jones, Leon K.
McLean, Darrell M.
Radebaugh, A. D.
Schmitz, Henry
Steenland, Adin P.
Warnock, Stephen J.
State College of Washington, Pullman
Apt, Walter J.
Burke, Douglas W.
Cummings, Robert H.
Fischer, George W.
Graham, Shirl O.
Halisky, Philip M.
Harris, Marion R.
Hendrix, J. Walter
Holton, Charles Stewart
Holtzman, O. V.
Locke, Seth Barton
Meiners, Jack P.
Shaw, Charles Gardner
Wright, Charles M.
Irrigation Experiment Station, Prosser
Barrat, Joseph G.
Blodgett, Earle C.
Diener, Theodor O.
Menzies, James D.
Twomey, James A.
Tree Fruit Experiment Station, Wenatchee
Kirkpatrick, Hugh C.
Lindner, Robert C.
Reeves, E. L.
Sprague, Roderick
Wolfe, Homer R.
Wright, Theodore R.
Western Washington Experiment Station, Puyallup
Campbell, Leo
Courtney, W. D.
Gould, Charles J.
Johnson, Folke

West Virginia

Craig, F. Waldo
Fulkerson, John F.
Johnson, Oscar H.
West Virginia University, Morgantown
Barnett, Horace L.
Bishop, C. Franklin
Elliott, Edward S.
Gallegly, Mannon E.
Jewell, Frederick E.
Leach, Julian G.
Orton, Clayton Roberts
Pristou, Robert
True, R. P.

Wisconsin

Anderson, A. Leroy
Bain, Henry F.
Brann, J. W.
Chambers, Ernest L.
Conant, George H.
Foll, S. C.
Hellman, Hans Petrus
Johann, Helen
Josephson, Leonard M.
Lindgren, Ralph M.
McDonough, E. S.
Peltier, George L.
University of Wisconsin, Madison
Allen, Oscar N.
Armolik, Neeme
Arny, Deane C.
Backus, Myron P.
Baldwin, Ira L.
Bancroft, John B.
Beckman, Carl H.
Berbee, John G.
Bloom, James R.
Boone, Donald M.
Cameron, H. Ronald
Chapman, R. Keith
Darling, H. M.
Dickson, James G.
Duffus, James E.

Fulton, Neil D.
Fulton, Robert W.
Gilbert, E. M.
Hagedorn, Donald J.
Hall, Dennis H.
Halpin, James E.
Hanson, Earle W.
Hildebrandt, Albert C.
Hoppe, Paul E.
Hougas, R. W.
Hung, Y. L.
Jones, Edward D.
Jones, Fred R.
Keitt, George W.
Kendrick, Edgar L.
Klemmer, H. W.
Kline, David M.
Kuntz, James E.
Lamey, H. A.
Larson, Russell H.
Lebeau, J. B.
Leben, Curt
Lindberg, G. Don
Lockwood, John L.
McDonald, W. C.
Moore, J. Duain
Muir, William H.
Parmeter, John R., Jr.
Patton, Robert F.
Paulus, Albert O.
Pelletier, Réal L.
Pierson, Charles F.
Pound, Glenn S.
Rieman, Gustav H.
Riker, A. J.
Ross, Arnold F.
Scheffer, Robert P.
Shands, H. L.
Shands, R. G.
Shea, Keith R.
Sinclair, James B.
Skotland, Calvin B.
Stahmann, Mark A.
Stowell, Ewell A.
Tsao, Peter H.
Van Arsdel, Eugene P.
Wade, Earl K.
Walker, John C.
Winstead, Nash N.

Wyoming

University of Wyoming, Laramie
Bridgmon, George H.
Peek, Chester A.
Schnathorst, William C.
Solheim, Wilhelm G.
Starr, George Herman
Walters, Hubert J.

Alaska

Coe, Donald M.

Hawaii

Sideris, Christos P.
Wismer, Chester A.
Pineapple Research Institute of Hawaii, Honolulu
Anderson, Earl J.
Carter, Walter
Sakimura, K.
Spiegelberg, Carl H.
University of Hawaii, Honolulu
Lohman, Marion L.
Murakishi, Harry H.
Sher, Samuel A.

Puerto Rico

Ayson, Amado A.
Bird, Julio
Bruehl, George W.
Hubert, Frederic P.
Lee, H. Atherton
Nolla, J. A. B.
Perez Otero, J. Enrique
Roure, Luis A.
Theis, Thomas N.

Guam

Liming, O. Neal

FOREIGN COUNTRIES

Argentina

Fischetti, D. F.
González, Ada M. Castronovo de
Hirschhorn, Elisa
Jauch, Clotilde

Australia

Baber, Esmond G.
Dickson, B. T.
Waterhouse, Walter L.
White, N. H.

Belgian Congo

Vekemans, Jacques

Belgium

Steyaert, Rene L.

Bolivia

Bell, Frank H.
Cardenas, Martín

Brazil

Bitancourt, Agesilau A.
Chaves Batista, A.
Costa, Alvaro S.
Drummond, O. A.
Gorenz, August M.
Guimaraes, F. F.
Jenkins, Anna E.
Kramer, Moyses
Machado, Adelmo A.
Rossetti, Victoria
Silberschmidt, Karl M.

Canada

Alberta
Broadfoot, William Craig
Cormack, M. W.
Davidson, Thomas R.
Henry, A. W.
Sanford, G. B.
Slykhuis, John T.
Tyner, L. E.
British Columbia
Foster, William R.
McIntosh, David L.
McKeen, W. E.
McLarty, Harold Ross
Newton, Margaret
Welsh, Maurice F.
Manitoba
Cherewick, W. J.
Gordon, William L.
Greaney, Frank J.
Green, Gordon J.
Hagborg, W. A. F.
Johnson, Thorvaldur
Machacek, John E.
Peturson, B.
Popp, W.
Sackston, W. E.
Wallace, Henry A. H.
Nova Scotia
Harrison, K. A.
Hockey, J. Fred
Ontario
Bailey, Dixon Lloyd
Berkeley, Garven H.
Conners, I. L.
Craigie, J. H.
Good, Harold M.
Graham, K. M.
Hanna, William F.
Hildebrand, Alexander A.
MacNeill, Blair H.
McKeen, Colin D.
Miller, J. J.
Racicot, H. N.
Sands, D. R.
Skolko, A. J.
Walker, Anson R.
Willison, Robert S.
Quebec
Blair, R. J.
Caron, Omer
Claude, (Father) M.
Havard, A. L.
Jacques, J. Emile
Langford, A. N.
Perrault, Champlain J.
Pomerleau, Rene
Saskatchewan
Russell, Ralph C.
Simmonds, Prye M.
Tinline, Robert D.
Vaartaja, L. Olli
Vanterpool, T. C.

Ceylon

Newton, William

Chili

Tartakowsky-Henker, Sergio

China

Loh, T. C.
Matsumoto, Takashi
Ou, Shu Huang
Tu, Chih

Colombia

Garcés O, Carlos
Rupert, Joseph A.
Skiles, Robert L.

Costa Rica

Carpenter, John B.
Flynn, J. E.
Imle, Ernest Paul
Langford, Michael H.
Loegering, William Q.
Orellana, R. G.
Sequeira, Luis

Cuba

Bryans, T. G.
Crandall, Bowen S.
Juantorena Dominguez, Julian M.
Morales Valcárcel, Federico G.
Summers, Eaton M.

Czechoslovakia

Peklo, Jaroslav

Denmark

Neergaard, Paul

Ecuador

Rorer, James Birch

Egypt

El-Helaly, Abbas Fathi
Warid, Warid A.
Yusel, Hasan M.

England

Bisby, Guy R.
Dickinson, Sydney
Fushtey, Stephen G.
Smith, Kenneth M.
Wilson, Alasdair R.

Finland

Hardh, J. E.

France

Morel, Georges M.
Viennot-Bourgin, G.
Zambettakis, Charalambos E.

Germany

Bremer, Hans

Greece

Critopoulos, P. D.

Guatemala

LeBeau, Francis J.
Lorenz, Rolland C.
Paddock, William C.

Honduras

Carpenter, Theodore R.
Hollis, John P.
Hord, H. H. V.
Stover, Robert H.
Thornton, Norwood C.

Hong Kong

Wei, C. T.

India

Capoor, Sant P.
Chona, B. L.
Lal, Trilok B.
Mishra, J. N.
Padmanabhan, S. Y.
Prasad, Nirankar
Raychaudhuri, S. P.
Rice, William N.
Shukla, T. N.
Thirumalachar, M. J.
Vasudeva, R. S.
Venkatakrishniah, N. S.

Israel

Littauer, Simon F.
Minz, G.

Italy

Ciccarone, Antonio
Ciferri, Raffaele
Goidànich, Gabriele
Hemerick, Glen A.
Ling, Lee
Sempio, Cesare

Japan

Asuyama, Hidefumi
Tochinai, Yoshihiko

Kenya

Gibson, Ian Alexander Scott
Storey, Harold Haydon

Mauritius

Wiehe, P. O.

Mexico

Borlaug, Norman E.
Campa-Pena, Carmen
Duffield, P. O.
Gibler, John W.
Niederhauser, John S.
Ortega, Benjamín C.
Remy, R. H.
Robles Gutierrez, Leonel
Wellhausen, Edwin J.
Yerkes, William D., Jr.

Netherlands

de Bruyn, Helena L. G.
Kerling, Louise C. P.
Mastenbroek, dr. ir. C.
Oort, A. Joan P.
Ten Houten, J. G.
Thung, T. H.

New Zealand

Watkins, Daniel Anthony

Nicaragua

Litzenberger, Samuel C.

Nigeria

Stanton, W. Robert

Norway

Heimbeck, (Mrs.) Louise Solberg
Jorstad, Ivar
Roll-Hansen, Finn

Pakistan

Aziz, Abdul
Hafiz, Abdul

Peru

Garcia-Rada, Germán
Postigo, Rosendo

Philippines

Clara, Feliciano M.
Park, Jai-Young F.

Salvador

Guiscafré-Arrillaga, Jaime

Scotland

Grainger, John

Switzerland

Gäumann, Ernst
Sinden, James W.

Union of South Africa

Bakker, E. M. Van Zinderen
Dippenaar, Barend J.
Rossouw, D. J.

Uruguay

Boasso, Celia

Venezuela

Gonzales Gorrondona, Alejandro
Malaguti, Gino
Marcano, Luis
Pontis-Videla, Rafael E.
Rodríguez Landaeta, Alejandro

CONSTITUTION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

As Revised to March 15, 1953

Article I—Name and Objectives

The name of this Society shall be The American Phytopathological Society. Its objects are to promote: 1) the increase and diffusion of all aspects of knowledge relating to plant diseases and their control, 2) effective working relations between persons professionally engaged in this field of study, and 3) coordination of various interests and services pertinent thereto.

Article II—Membership

Section 1. The Society shall consist of Charter Members, Annual Members, Student Members, Patrons, Sustaining Associates, and Emeritus Members.

Section 2.

(a) *Charter Members.* The Charter Members are the one hundred thirty persons who accepted the invitation of the Organization Committee of October 25, 1909, to form the Society.

(b) *Annual Members.* Any person interested in the study and control of plant diseases shall be eligible for membership. Applications for membership must be endorsed by at least one member of the Society and forwarded to the treasurer with such dues as may be prescribed in the Standing Rules. Applicants may be elected at any regular meeting of the Society or by the Council in the interim.

(c) *Student Members.* Any person interested in the study and control of plant diseases who is enrolled as a senior or graduate student in an accredited college or university is eligible for student membership for a period not to exceed five years provided he is not gainfully employed more than two-thirds time. Applications must be endorsed by a member of the Society, and the student and employment status must be certified by the major professor or appropriate department head of the educational institution. Student Members are accorded all the tenure and privileges of Annual Members but must renew the student certification each year when submitting to the treasurer such dues as may be prescribed in the Standing Rules, until they complete their academic training or become employed more than two-thirds time when they automatically become eligible for regular Annual Membership. Applicants may be elected at any regular meeting of the Society or by the Council in the interim.

(d) *Patrons.* Any person making a payment to the Society of such amount as may be prescribed in the Standing Rules shall be designated a Patron and, upon election to membership, shall have all the privileges of an Annual Member.

(e) *Sustaining Associates.* Any firm making an annual contribution to the Society of such amount as prescribed in the Standing Rules shall be designated a Sustaining Associate, and shall receive the official journal without charge.

(f) *Emeritus Members.* Members of twenty years' standing may become Emeritus Members upon application to the Secretary. An Emeritus Member shall retain all rights and privileges of the Society and is exempt from payment of dues, but shall not receive Phytopathology unless he so desires, in which case he shall receive it at cost.

Article III—Officers

Section 1. The officers of the Society shall be a President, a Vice President, a Secretary, and a Treasurer.

Section 2. The duties of the officers shall be those customarily pertaining to these offices.

Article IV—Council

Section 1. The government of the Society and its corporate authority as defined in the Society's Articles of Incorporation shall be vested in a Council which shall consist of the contemporary officers, the retiring President, the President-elect, and the Editor-in-Chief and the Business Manager of the Journal, Phytopathology, a Councilor from each unit of the Society that is organized as a Division, and two Councilors-at-Large.

Section 2. The President shall call meetings of the Council at his discretion or upon the written request of three of its members. He, or in his absence the Vice President, shall preside at meetings of the Council. In the absence of both the President and Vice President, the President-elect shall preside at meetings of the Council.

Section 3. The presence of a majority of the Council shall constitute a quorum for the transaction of business.

Section 4. All actions of the Council or officers must be authorized or approved by the Society at the annual meeting except as specified in Article VII, Section 3.

Article V—Selection of Officers and Councilors

Section 1. The President-Elect, Vice President, and Councilors-at-Large shall be elected by ballot. The Secretary shall send a nomination ballot for these offices to all members of the Society in time to allow nominations to be returned not less than two months before the date of the annual meeting. The Council shall designate the nominees for any office when such nominations are wanting, or are tied. The names of the three persons who receive the highest number of nominating votes for each office shall be placed on a final ballot, which shall be sent to each resident member not less than one month before the annual meeting. If the same person should receive sufficient ballots to qualify for nomination to more than one office, his name shall be placed on the final ballot as a candidate for only the office for which he receives

the greater number of ballots, and in the case of an equal number of ballots he shall be entered as a candidate for only the higher office as listed in Article III, Section 1. These ballots shall be returned to the Secretary and shall be canvassed by the Council. A plurality vote shall elect.

Section 2. The Secretary and the Treasurer shall be appointed by the Council, ordinarily for terms of three years, which shall not expire concurrently, and the Council may adjust the term or the date of assuming office to avoid this contingency.

Section 3. The President-elect shall become President and assume the duties of the office upon final adjournment of the annual meeting next succeeding the annual meeting at which he is elected. The Vice President shall assume office upon final adjournment of the annual meeting at which he is elected. The President and Vice President shall not be eligible to immediate reelection to the same offices.

Section 4. The terms of the Councilors-at-Large shall be two years beginning with the final adjournment of the annual meeting at which they are elected, or until their successors are elected.

Section 5. Division Councilors shall be elected by their respective Divisions in such manner and for such terms as the Divisions may prescribe.

Section 6. The Council shall fill by appointment any vacancy, except that of a Division Councilor, occurring within the prescribed term, such appointment to continue for the unexpired balance.

Section 7. The President shall appoint Alternates-at-Large for the Councilors-at-Large who are unable to attend the official Council meetings.

Article VI—Society Journal and Editorial Board

Section 1. The official publication of the Society shall be the journal PHYTOPATHOLOGY.

Section 2. The Council shall appoint an Editor-in-Chief and a Business Manager of the Journal for terms of three years not expiring concurrently, and also an Editorial Board as prescribed in the Standing Rules.

Section 3. The Council may authorize the Editor-in-Chief and the Business Manager to employ such assistants as may be necessary for the proper conduct of their work.

Article VII—Committees and Conduct of Society Business

Section 1. The Council shall appoint such standing and special committees as may be appropriate for conducting the business of the Society.

Section 2.

(a) *Standing Committees.* Committees whose functions include the general policies and internal relations of the Society, and its relations with other organizations, shall be known as Standing Committees. They shall have a revolving membership and shall submit an annual report to the Council at the Society's annual meeting.

(b) *Special Committees.* Committees whose function is to deal with special subjects of concern to the Society shall be designated Special Committees. Each special committee shall be continued for such period as in the judgement of the Council may be necessary for the accomplishment of its purpose. Interim reports may be requested and a final report shall be made to the Council at the close of this period.

(c) *Temporary Committees.* Committees for the accomplishment of a specific purpose, limited in scope and time, may be appointed by the President to serve during his term and to make such reports to the Council as he may direct.

Section 3. In the interim between regular meetings of the Society the Council may undertake and carry out such actions as it deems advisable or necessary, including the reference of particular questions to the Society members by mail or by notices in the official Journal, subject to approval by the Society at its next meeting.

Article VIII—Funds

The control of funds of the Society received from dues, subscriptions to the Journal, gifts, bequests and endowments shall be vested in the Council to administer through the appropriate fiscal officers and committees. An audit of the receipts and disbursements shall be made annually by a temporary committee, or at the discretion of the Council by a certified public accountant. The reports of the Treasurer and the Business Manager, together with that of the auditing committee, shall be published annually in the official journal.

Article IX—Meetings

A general meeting of the Society shall be held each year, unless prevented by a national emergency, at such place, and time as the Council may direct. Special or local meetings for the presentation of scientific papers or demonstrations of experimental results may be arranged at the discretion of the Council.

Article X—Divisions

Section 1. Branch organizations or units within the Society, known as Divisions, may be established within the geographical area of North America, provided that a formal application setting forth the reasons for such establishment is made to, and approved by, the Society.

Section 2. The organizations and the operations of Divisions shall be governed by the specifications relative to Divisions in the Standing Rules.

Article XI—Affiliated Societies

For the purpose of promoting international collaboration between professional organizations in the field of plant pathology, the Council of The American Phytopathological Society is authorized to establish affiliated relations with other phytopathological socie-

ties in accordance with the provisions pertaining thereto in the Standing Rules.

Article XII—Ratification and Amendment

Section 1. This Constitution shall become effective upon its ratification in accordance with the procedure for amending the original Constitution of the Society. It shall supersede the original Constitution and all amendments thereto.

Section 2. This Constitution may be amended at any annual meeting of the Society provided that any proposed amendment be approved by the Council and be published in the official journal, or otherwise communicated to all resident members, at least one month before the annual meeting, and that it receive the affirmative votes of three-fourths of the members voting at a regularly scheduled business session.

Standing Rules

1. dues, arrears, reinstatement

(a) The dues, including subscription to Phytopathology, for all Annual Members as defined in Article II, Section 2 (b) of the Constitution shall be $10.00 a year, and for Student Members, as defined in Section 2 (c), shall be $5.00 a year. In order to qualify for the rate as a Student Member, applicants must submit with their dues each year a certificate of student status endorsed by their major professor or the head of the department of instruction.

(b) All dues shall be payable by December 31, and those who fail to make payment to the treasurer by this date shall be considered in arrears and dropped from membership. Members in arrears may be reinstated, without the formality of reelection, by payment of current dues and, in the case of students, certification of student status as specified in Section 2 (c) of Article II of the Constitution.

(c) The contribution of a Patron shall be one thousand dollars, payable in one sum.

(d) The annual contribution of a Sustaining Associate shall be at least one hundred dollars, and a list of Sustaining Associates shall be published in each issue of the journal.

(e) The payments received from Patrons and Sustaining Associates shall be credited to the current funds of the Society. These funds shall be transferable to the account of Phytopathology, or may be used for other purposes authorized by the Council.

2. phytopathology

(a) *Editorial Board.* The editorial policy of the official journal, Phytopathology, shall be vested in an Editorial Board consisting of an Editor-in-Chief, three Editors, and twelve Associate Editors; the business control shall be vested in a Business Manager and an Advertising (or Assistant Business) Manager.

(b) *Selection of Board Members.* This Board, aside from the Editor-in-Chief and the Business Manager, the manner of whose appointment is prescribed in Article VI, Section 2 of the Constitution, shall be selected from the membership by the Council in consultation with the Editor-in-Chief, and approved by the Society.

(c) *Terms of Office.* The term of each member of the Board shall be three years, provided that the terms of the three Editors shall not be concurrent, and that four Associate Editors shall be appointed each year. The Council is authorized to make any adjustments in these terms of office necessary to effect non-concurrence.

(d) *Compensation of Advertising Manager.* The Advertising Manager may receive compensation for his services on a commission basis as determined by the Council.

(e) *Subscriptions and Back Numbers.* Subscriptions to Phytopathology shall be $10.00 per year, domestic and foreign. The price of single current numbers, within 60 days of publication, shall be $1.00. The sale and price of back volumes or numbers shall be determined by the Business Manager with the approval of the Council. Requests to supply lost copies of the journal without charge must be made within sixty days from date of issue. (January 15 and monthly thereafter).

(f) *Income from advertising.* Income received from the sale of advertising space in Phytopathology shall be devoted to the production of the journal.

3. program committee

The program for the annual meeting shall be in charge of a temporary committee consisting of the President, Vice President, Secretary, Editor-in-Chief, and such additional members as the President may select. This committee shall have full authority over the scheduling of sessions and demonstrations, and the allocation of papers.

4. expenses of officers at annual meeting

The Secretary, the Treasurer, the President or in his place the Vice President, are authorized to receive reimbursement from the Society for travel expenses in connection with their attendance at the annual meeting. In addition the Editor-in-Chief of Phytopathology is also authorized to receive reimbursement for travel expenses to the annual meeting.

5. auditing committee

An auditing committee shall be appointed by the President each year which shall, prior to the annual meeting, audit the accounts of the Treasurer and the Business Manager, and certify their audit in a written report to the Society at the final business session. The Council may at its discretion authorize an audit by a certified public accountant as well as an auditing committee. Such audit shall be published in the official journal.

6. submission of papers and abstracts

(a) Three typewritten copies of each abstract of a

paper to be presented at the annual meeting must be submitted to the Secretary on or before the closing date specified in the Preliminary Announcement. This date shall be set by the Secretary and when possible shall be sixty days prior to the annual meeting. The Secretary shall return to the authors all abstracts received after the closing date.

(b) At least one author of each paper must be a member of the Society. An author cannot appear on more than two papers.

(c) The abstracts must be in clear, concise English and contain no tabular data. The text must not exceed two hundred words in length. Only unpublished information may be included. Reports of progress, or of disease occurrence, or of routine tests, or of ordinary control measures are not desired unless new and significant developments are clearly indicated.

(d) The abstracts shall be subject to final acceptance and editing by a reviewing committee appointed by the Editor-in-Chief. Accepted abstracts will be published in PHYTOPATHOLOGY at the expense of the Society, provided that not more than two abstracts under the sole or joint authorship of one member, that are presented at the meetings of the Society or the Divisions, shall be published at the Society's expense within one calendar year.

(e) All copies of the abstract must have the following information 1) title of paper, 2) name, address, affiliation and membership status of each author, 3) sectional preference, 4) time required for presentation (maximum 15 minutes), 5) projection equipment required, including slide size.

(f) Abstracts of papers invited by the Program Committee, or submitted to other societies for inclusion in joint programs will be published in PHYTOPATHOLOGY provided they meet the above requirements, except that the rules regarding unpublished data and membership in the Society will be waived.

7. DIVISIONS

The following provisions shall govern the organization and regulation of Divisions of the Society.

(a) *Name.* Divisions shall use the name of the parent Society with appropriate geographical term; for example, The American Phytopathological Society, Pacific Division.

(b) *Membership.* Divisions shall elect to full membership only members of The American Phytopathological Society, but each Division may elect associate members under such rules as it may adopt.

(c) *Publication.* Summaries of the proceedings of the annual meetings of Divisions shall be published in PHYTOPATHOLOGY at the Society's expense. Abstracts of papers presented at Division meetings also may be published in PHYTOPATHOLOGY subject to the rules governing the preparation and publication of abstracts as stated in Rule 6, and with the specific limit of two on the number of abstracts that any member may have published at the Society's expense within one calendar year.

(d) *Constitution.* The constitution or articles of organization of all Divisions shall contain a provision or provisions ratifying the above rules. The constitution of all Divisions shall contain nothing in conflict with the constitution of The American Phytopathological Society. With the exceptions defined by the above rules, the Divisions shall enjoy complete autonomy.

8. AFFILIATED SOCIETIES

The following provisions shall govern the establishment of affiliated relations with other phytopathological societies.

(a) Upon advice to the Council that affiliation is desired, the Council may invite to affiliation with The American Phytopathological Society any other professional society in the field of plant pathology.

(b) The existence of affiliation of other societies with this Society shall be indicated by listing the names of affiliated societies in each number of PHYTOPATHOLOGY.

(c) The Council may at its discretion authorize the publication of summaries of the proceedings of annual meetings of affiliated societies in PHYTOPATHOLOGY.

(d) The Council may at its discretion authorize the publication for limited periods, subject to annual renewal of the privilege, of research papers of members of affiliated societies who are not members of The American Phytopathological Society.

9. SOCIETY REPRESENTATIVES IN OTHER ORGANIZATIONS

The Council is authorized to select, with the approval of the Society, representatives in other organizations in which the Society is entitled to representation. The following are specifically authorized: two representatives on the Council of the American Association for the Advancement of Science for a two-year term; one representative on the Division of Biology and Agriculture of the National Research Council for a three-year term; one member of the Editorial Board of the American Journal of Botany for a three-year term; and one representative on the Board of Governors of the American Institute of Biological Sciences for a term of four years.

10. AMENDMENTS

These rules may be amended by a majority vote of the members voting at any regular meeting of the Society, or by a majority vote of the members voting in a ballot conducted by mail.

INDEX FOR VOLUME 43

AUTHOR AND SUBJECT INDEX. New species and varieties in bold face type. Junior authorship indicated by pages in parentheses.

Abaca sheath rot, (*Marasmius stenophyllus*) susceptibility and control, 479
ABBOTT, E. V., 289
Acer macrophyllum, apple flyspeck host, 586
Acidity, see Hydrogen-ion concentration
Actidione, see under Antibiotics
Actinomycete, gummosis of grapefruit, associated with, 101
ADAM, ANGELOS, 465
ADAMS, D. F., (587)
ADAMS, ROBERT E., 465
Adenine, effect on rooting of *Prunus mahaleb*, and effect of pretreatment on effect of, 587
ADP, 224
Aegilops triuncialis, 310
African violet, root-knot nematode, 406
Agallia constricta, see under Insects
Agalliopsis novella, see under Insects
Agaricaceae, wood rotting by, 21
Agrilus bilineatus, see under Insects
Agrobacterium tumefaciens,
  colonial variants, morphology, 509
    pathogenicity and response to biochemical tests, 510
    stability in galls and culture, 510
Agropyron trachycaulum, 309
AKELEY, ROBERT V., (245)
Albugo candida, overwintering, 485
Aldehyde, effect on virulence of Pythium root-rot pathogen, 289
Alfalfa black stem, 378
    control, fungicides, 379
    effect on seed crop in field and greenhouse tests, 378
    effect on seed germination, 381
    measurement of seed loss from, 380
  crown rot, hydrogen cyanide production by pathogen, effect of temperature and media compared, 581
  leaf spot, resistance, classification of plants for, 653
    microscopic evidences of resistance, 652
    rapid method of measurement of resistance, 651
    Pseudoplea, Stemphylium botryosum as perfect stage, 584
    Stemphylium, 477
  mosaic virus, complex, 271
    strain, age of plant and method of inoculation, 473
    strains, 275
      differentiation, 479
    tomato varieties, 479
  nurse plant for leafhopper vectors of wound-tumor virus, 387
  Pierce's disease, grape degeneration on, 611
    yellow-headed sharpshooter vector, 612
  Pythium spp. pathogenicity on seedlings, 474
  root-rot, Phytophthora, 586
    tests for control, 589
  winter crown rot, HCN production by pathogen, 478
    host parasite relation, 478
  wound-tumor virus, insect vectors, 9
  yellow mosaic virus (*Marmor medicaginis* H. var. **flavovarians** n. var.) on, 38
ALLEN, PAUL J., 221
ALLEN, ROSS M., 585
Allium cepa, see Onion
Almond, *Sclerotinia fructicola*, and *S. laxa*, pathogenicity compared, 589
Alternaria porri, onion, effect on in storage, 411
    onion purple and brown blotch, 409
  solani, inhibition by phosphate buffer, 478
    nutrition of host, effect on susceptibility to, 476
    toximycin as protectant spray, 25
  sp., in bean seeds, 483
Alternaria—*continued*
    spp., on peanuts in market, 474
      on soybean, 292
    tenuis and *tenuissima*, 409, 411
Althaea rosea, see hollyhock
Amaryllis, spiral nematode, 297
American Phytopathological Society:
  abstracts for the 45th annual meeting (Madison), 465
  analysis of membership, D1
  charter members, D4
  classes of membership, D1
  constitution, D55
  directory of members, D10
  divisions, D2
  fields of interest reported by members, D3
  geographical index of members, D46
  growth, D1
  in memorium, D6
  introduction to directory, D1
  organization, 1953, D9
  past councilors, D7
  past editors-in-chief, D7
  past officers, D7
  professions and occupations of members, D2
  report of 44th annual meeting, 52
  reports and abstracts:
    Northeastern Division, 108
    Pacific Division, 585
    Potomac Division, 404
    Southern Division, 288
  source of employment of members, D2
  sustaining associates, D10
Amino acids, see also under Nutrition of microorganisms
  carnation bacterial wilt studies, 587
  effect on melanin production and growth in *Streptomyces scabies*, 357
  production by *Ustilago zeae* in culture, 470
Ammate, wheat stem rust, 661
ANDERSON, E. J., 465
ANDERSON, H. W., (465)
Andropogon barbinodis, 310
Annulus, see also Viruses
  rhubarb ringspot-like virus, 590
Antagonisms, *Fusarium vasinfectum*, soil microorganisms, 482
  Penicillium soil isolate to fungi, 479
  soil microflora, *Sclerotium rolfsii*, 366
Anthoxanthum odoratum, 310
Anthracnose, oak, 103
  watermelon, breeding for resistance to, 254
Antibiotic production, by *Aspergillus clavatus* and *Penicillium patulum*, 108
Antibiotics, actidione, 486
    bordeaux compared, cherry leaf spot, 468
    cherry leaf spot, 109, 468
    Curvularia "fading-out" on turf, 109
    lime, effect upon effectiveness, 109
    methoxychlor, compatible with, 109
    orthocide mixture, wheat covered smut, 461
    pH effect on activity, wheat rusts, 498
    Rhizoctonia, 475
    wheat leaf and stem rust, control and phytotoxicity, 493
  antimycin A,
    A-102, A-35 composition compared, 478
      oat seedling blight, 478
      produced by new isolate of *Streptomyces* sp., 478
      tomato early blight protectant, 478
    effect on respiration, 227
  aureomycin, oxidative phosphorylations, action on, 225
    respiration, effect on, 221
    Verticillium, 475
  bacitracin, *Rhizoctonia*, 475

Antibiotics—*continued*
chloramphenicol, effect of concentration in medium on synthesis by *Streptomyces venezuelae*, 478
chloromycetin, *Rhizoctonia*, 475
gramicidin, oxidative phosphorylations, action on, 225
respiration, effect on, 221
Staphylococcus aureus, reaction to, 225
helixin B, action across air space on *Glomerella cingulata* spores, 393
barley seedling blight, field and greenhouse tests, 392
oat Helminthosporium blight in field and greenhouse tests, 391
seed treatment, small grain, 391
inhibition of, 233
magnamycin, *Rhizoctonia* and *Verticillium*, 475
naturally occurring, 226
patulin and vivotoxins, 233
penicillin, *Rhizoctonia*, 475
polymixin, *Rhizoctonia* and *Verticillium*, 475
potato late blight effect on, 463
respiration, effect on, in microorganisms, 225
in plant tissue, 221
rimocidin, potato late blight, 464
Rhizoctonia, 475
streptomycin, bean halo blight, 407, 480
effect on Bartlett pear seedlings, 405
effect on Elberta peach, 405
effect on respiration, 227
in isolation of *Fusarium* spp. from plant tissues, 700
movement in bean, 480
Rhizoctonia and *Verticillium*, 475
terramycin, effect of concentration in medium upon synthesis by *Streptomyces venezuelae*, 478
effect on Elberta peach, 405
peach leaf drop, 405
potato late blight, 464
Verticillium, 475
thiolutin, potato late blight, 464
Rhizoctonia and *Verticillium*, 475
toximycin, antifungal, from *Bacillus subtilis*, 23
usnic acid, oxidative phosphorylations, action on, 225
respiration, effect on, 221
Antimycin, see under Antibiotics
Antirrhinum majus, see Snapdragon
Aphanomyces cochlioides, sugar beet seedlings, black rot, 300
Aphids, see under Insects
Apium graveolens, see also Celery
bayberry-yellows virus on, 18
western mosaic aphid transmission, 29
APPLE, J. L., 289
Apple, black rot, effect of continual use of fungicides, 268
Botryosphaeria rot pathogens, susceptible apple varieties, 407
disease control, mist and hydraulic spraying compared, 465
Dothiorella spp. associated with cankered branches, 407
fluoride concentrations, foliar, factors affecting, 587
flyspeck, ecology and host range, 586
perfect and imperfect stage of pathogen, 470
scab, control by fungicide mixtures, 109
seedlings in greenhouse, testing of fungicides on, 109
Apricot, *Sclerotinia fructicola* and *S. laxa*, pathogenicity compared, 589
Arachis hypogaea, see Peanut
ARAGAKI, M., (468), 643
Arbutus menziesii, apple flyspeck host, 586
Arctium minus, *Verticillium albo-atrum* on, 588
Aristida oligantha, 310
Armillaria mellea, culture studies, 482
wilt-inducing pigment from, 486
ARMSTRONG, G. M., 465, (465)
ARMSTRONG, J. K., (465), 465
ARNDT, C. H., 220
ARNY, D. C., (391), 465
Arthrobotrys sp., nematode capture by, 405
Ascochyta abelmoschi, in culture, 293
on lupine, 294
phaseolorum and *gossypii* compared, host range, 469
temperature, optimum for growth, 294
gossypii, *abelmoschi* and *phaseolorum* compared, host range, 469
cotton dusting for control, 292
temperature, optimum for growth, 294
imperfecta, alfalfa, effect on seed crop in field and greenhouse tests, 378
fungicide control tests, 379
host range, 378
phaseolorum, *abelmoschi* and *gossypii* compared, host range, 469
spp., cross-inoculations, okra, bean and cotton, 469
ASHRAF, MUHAMMAD, (580)
Aspen, white heartrot, 699
Aspergillus clavatus, antibiotic production in soil and manures, 108
niger, on soybean, 292
spp., on peanuts in market, 474
ATKINSON, D., (587)
ATP, 224
Aureogenus magnivena, *Agalliopsis novella*, transovarian passage in, 9
vastans, *Agallia constricta*, transovarian passage in, 10
Aureomycin, see under Antibiotics
Autogenous necrosis, cabbage, 415
Autoradiography, detection and assay of radioactive toxins *in vivo*, 237
Avena barbata, 310
Avocado, Phytophthora root rot resistance, 490
AYCOCK, ROBERT, 50
Azalea, dagger nematode, 296

Bacillus phytophthorus, effect on respiration of potato, 222
polymyxa, effect of, on tissue culture, 92
spp., in seeds of certified beans, 483
pepper fruit spot disease in Palestine, 92
soft rot in various vegetables, 92
subtilis, antibiotic, toximycin from, 23
effect of, on tissue culture, 92
Bacitracin, see under Antibiotics
Bacteria, ring-rot on potato bags, ultra-violet, X-ray and nuclear radiation, effect of, on, 440
Bacterial blight, chrysanthemum, 522
soybean, inoculum dilution and host reaction, 468
pathogen in seed and soil, 189
canker, tomato control, 473
leaf spot pathogens association with soybean seed, soil and debris, 189
metabolites, effect of on carrot tissue, 92
pustule, soybean, pathogen in seed and soil, 189
spot, broccoli, 583
spring canker, peach seasonal occurrence, 465
stem rot, geranium, 588
wilt, carnation, cause of wilting, 587
carnation, virulence, retention in culture, 406
tobacco, 240
Bacteriophage, *Xanthomonas pruni*, factors affecting plaque formation, 27
BAINES, R. C., 465
BAKER, KENNETH F., (470), 585
BALD, J. G., 141, 146, 151, 167, 585, (587)
Barberry, Japanese, cucumber mosaic virus host, 489
Barley, cereal yellow-dwarf virus, aphid vectors, 309, 312
epiphytotic conditions favoring, 310
effect of air temperature and nitrogen supply on injury by olefinic peroxide fumigants, 588
ergot associated with loose smut, 461
Helminthosporium spp., reactions to isolates of, 517
powdery mildew, environmental conditioning of plants, effects on susceptibility to, 162

Barley—*continued*
Pythium graminicola, varieties resistant to, 474
seedling blight control, 391
Septoria leaf blotch, host-parasite relationships, 473
smut, covered, control, 393
inoculating method, 697
loose, control, 393, 313
ergot associated with, 461
races, 407, 482
resistance of crosses to races of, 371
viability of seeds of parental varieties and crosses inoculated with pathogen, 370
varieties, differential test plants for loose smut, 407
wheat striate mosaic virus on, 539
yellow-dwarf virus, 128
effect on yield, 134
BARNETT, H. L., 341
BARRUS, M. F., 350
BART, GEORGE J., 466
Basidiobolus spp., odor of benzene hexachloride and of *Streptomyces* spp. produced by, 405
Basidiomycete, alfalfa winter crown rot, HCN production by, 478
host parasite relation, 478
species, hydrogen cyanide production, alfalfa crown rot, and effect of temperature and media, 581
BASSETT, E. G., (110)
BAXTER, JOHN W., 178
BAXTER, L. W., 466
Bayberry-yellows virus, transmission, 15
Bean, alfalfa black stem on, 378
mosaic strains on, 271
yellow mosaic virus on, 38
anthracnose, pressure effects on, 70
Ascochyta spp. on, 469
broad, cucumber mosaic virus strains on, 334
clover-root nematode on, 606
cucumber mosaic virus strains on, 334
halo blight, control, streptomycin, 407, 480
lima, Cercospora purple stain, seed, 477
cucumber mosaic virus strains on, 334
downy mildew, 419
potassium nutrition, relation to root-knot nematode development in, 171
root-knot nematode on, 171
microorganisms in certified seeds of, 483
Mung, alfalfa black stem on, 378
olefinic peroxide leaf injury and protectants, 588
powdery mildew, control in laboratory, 477
pressure effects on, 70
rice, alfalfa black stem on, 378
cucumber mosaic virus strains on, 334
root rot control, soil fumigation, 590
rust, pressure effects on, 70
seed, orange color caused by *Coynebacterium flaccumfaciens*, strain of, 483
snap, Cercospora purple stain, seed, 477
southern mosaic virus, serological test for, 77
sting nematode, 295
streptomycin movement in, 480
Tepary, cucumber mosaic virus strains on, 334
tobacco mosaic virus, acquired resistance, 490
necrosis virus strain, adapted to, 590
velvet, alfalfa black stem on, 378
white mold, occurrence in Idaho, 519
yellow mosaic virus, age of plant and method of inoculation, 473
necrotic-lesion producing strain (new) on, 45
strains on, 271
BECKMAN, C. H., 441, 448
Beet, seedling necrosis, fungicide in soil and fertilizer tests, 590
stubby-root nematode, 296
Belonolaimus gracilis, see also Nematodes, sting
control, 291, 438
on various crops, 291, 434
2-benzothiazolylthioglycolate, sodium and potassium salts of, formative effects on tomato, effects on chemical composition on tomato plants, in chemotherapy of Fusarium wilt, 43
BERAHA, LOUIS, 383, 466
BERBEE, FLORA, (466)
BERBEE, J. G., (448), 466
Berberis Thunbergii, see Barberry, Japanese, 489
BERGESON, G. B., (264)
BERKELEY, G. H., 111
Beta vulgaris, see also Beet, bayberry yellows virus, 18
BEVER, WAYNE M., 681
Biographical sketch, Forest Milo Blodgett, 350
E. W. Bodine, 60
Luther Parris Byars, 59
Melville Thurston Cook, 591
Richard English Vaughan, 349
James Stewart Wiant, 592
Biological control, possible explanation of, 701
Birch yellow, canker and decay, *Poria laevigata*, incitant, 487
BIRCHFIELD, WRAY, 289
Birdsfoot trefoil, *Stemophylium* loti sp. nov., 577
BLACK, L. M., 9, (387), 466
Black medic, alfalfa black stem on, 378
red clover vein-mosaic virus, 473
Black root rot, tobacco resistance to, 239
Black rot, apple, effect of continual use of fungicides, 268
sweet potato, differences in susceptibility of varieties to, 78
Black shank, tobacco, resistance to, 239
BLOCH, ROBERT, 351
Blodgett, Forest Milo, Biographical sketch, 350
BLOOM, JAMES R., 466
Blueberry, blossom and twig blight control, 481
stem canker, resistant varieties, 589
Bodine, E. W., Biographical sketch, 60
BOLTON, A. T., (345)
BONDE, REINER, 463
BOOSALIS, M. G., (401), 467
BOOTHROYD, C. W., 467
BORLAUG, NORMAN E., 467, (468)
Botryodiplodia phaseoli, *Macrophomina phaseoli* as synonym, 610
Botryosphaeria ribis, apple rot in Maryland, 407
muscadine grape, 629
shell bark relation to, 467
Botrytis cinerea, blueberry blossom and twig blight control, 481
methyl bromide control, 376
potato, effect of maturity and water content of tubers on susceptibility to, 338, 339
effect of peptic enzymes on, 340
shell bark relation to, 467
strawberry gray mold control, 482
gladiolorum, control, 151, 154
corm lesions, 146
heat-curing of corms infected by, 141
neck rot humidity, effect on, 169
host tissue defensive reactions, 170
leaf spot, comparison with, 167
origin of, 167
soil temperature, effect on, 169
sporulation, 169
spp., onion blast incitants, 483
Bouchea prismatica, spot anthracnose, 406
BOUCHEREAU, PAUL E., 289
Bouteloua cupitpendula, 310
BOYCE, JOHN S., JR., 644
BOYLE, JOHN S., 467
BOYLE, LYTTON W., 571
BRAKKE, MYRON K., 387, 467

BRANDT, WILLIAM H., 467
BRANN, J. L., (480)
Brassica juncea, B. nigra virus property studies, 541
green peach aphid virus transmission, 209
nigra, virus, aphid-host plant interaction variation, 209
aphid transmission, 209, 254
host range, 541
properties: thermal inactivation, dilution, end-point, longevity *in vitro*, 541
oleracea, see Cabbage
BRAUN, ARMIN C., 204
BRENER, W. H., (466)
BRIERLEY, PHILIP, 404 (406)
Broccoli bacterial spot, *Pseudomonas maculicola*, incitant, 583
Bromus spp., 310
Brown patch, susceptibility of various lawn grasses to, 110
Brussels sprouts, *Heterodera cruciferae* on, 260
Buchloe dactyloides. see under Grasses, buffalo
Buffer systems, tomato early blight control and fungus inhibition, 478
Buprestidae, see under Insects
BURKHOLDER, W. H., (350), 522
Butterfly pea, alfalfa black stem on, 378
Byars, Luther Parris, Biographical sketch, 59

Cabbage, autogenous necrosis, inheritance of, 415
Badger Market, new disease resistant variety, 649
black ring virus, effect of air temperatures on, in *Nicotiana* spp. 550
Fusarium, metabolites production of vascular browning, 490
stubby-root nematode, 296
virus A, effect of air and soil temperature on, in *Nicotiana* spp., 550
yellows, 278, 650
resistance, basis of, 489
Caeoma spp., nomenclature, changes in, 183
CAIRNS, ELDON J., 105, 404
Cajanus indicus, see Pigeon pea
CALAVAN, E. C., 467
Calyptospora columnaris, 360
Camellia, spp., *Glomerella cingulata* on varieties of, 466
leaf and flower variegation, graft transmitted, 293
CAMPANA, RICHARD J., 468
CAMPOS, T. A., 468
Cantaloupe, cucumber Fusarium on, 481
Capsicum, see under Pepper
Captan, see also under Fungicides, 58
Carbon^{14}, see Radioisotopes
Carnation, bacterial wilt, cause of wilting, 587
clover-root nematode on, 606
fumigant injury, 489
Fusarium stem rot, 485
greasy blotch, 585
Carneocephala flaviceps, see Insects, yellow-headed sharpshooter
CARPENTER, T. R., 156
Carrot, bacterial metabolites, effect of on tissue culture, 92
tissue culture, effect of bacteria on, 92
CARTER, J. C., 468
Caryophyllaceae, carnation Fusarium wilt, susceptibility to, 465
Castanea spp., see Chestnut
CATION, DONALD, 468, (471)
Celery, cucumber mosaic virus strains on, 336
disease resistance, breeding for, 254
seedbed disease control, 293
sting nematode, 293, 295
stubby-root nematode, 296
western mosaic, aphid transmission, 29
CENOZ, HUGO P., (487)
Cerambycidae, see under Insects
Ceratocystis fimbriata, black cane rot of *Syngonium auritum*, form of, 586
Cercospora apii, celery, resistance, 254
beticola, sugar beet breeding for resistance to, 298
seminalis, on buffalo grass, etiology of, 488
spp, on soybean, 292
soybean seeds, purple strain, 477
Cereal yellow-dwarf virus, see under Viruses
Cerosporella spp., nomenclature, changes in, 183
CERVANTES, JAVIER, (480)
CETAS, R. C., (467)
Chaetomium spp., in soil, inhibitory to damping-off of peppers, 586
Chalara fagacearum, association with other wood rotting fungi, 467
oak wood, effect on, 467
quercina, see also *Endoconidiophora fagacearum* carbon, sources for growth in culture, 445
heterothallism, 67
insects associated with, 69
nitrogen sources for growth in culture, 444
oxidizing and reducing agents effect on growth in culture, 442
pathogenicity, 68
perithecial stage of, 65
pH effect on growth in culture, 442
vitamins and purines for growth in culture, 443
CHAMBERLAIN, D. W., 468
CHANDLER, W. A., 108
Charcoal rot, effect of temperature and moisture on growth, 634
linear growth rates of the fungus, 633
pycnidial stage of fungus, 608
Chayotes, stubby-root nematode, 296
Chemotherapeutants, benzothiazole, 44
2-benzothiazolythioglycolic acid, derivatives, Dutch elm disease, 486
potassium and sodium salts of, comparison of fungitoxic and chemotherapeutic potency, 44
calcium sulfamate, pH effect on activity, wheat rusts, 498
wheat leaf and stem rust control, 497
4-carbomethylaminophenyl sodium sulfonate, wheat leaf and stem rust control and phytotoxicity, 497
2,4-dichlorophenoxyacetic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
3,5-dinitrobenzoic acid, wheat stem rust, 661
ferbam, tobacco blue mold, 486
hydrazines, wheat leaf and stem rust control and phytotoxicity, 497
indole-3-acetic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
mercaptobenzothiazole, 44
α-naphthaleneacetic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
α-naphthoxyacetic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
nitrated and hydroxy benzine derivatives, wheat stem rust, 662
oxyquinoline benzoate and sulfate, oak wilt, 475
phenylhydrazine, pH effect on activity, wheat rusts, 498
wheat leaf and stem rust, control and phytotoxicity, 497
picric acid, wheat stem rust, 661
potassium α(2-benzothiazolyl) thiopropionate, potassium β(2-benzothiazolyl) thiopropionate, 44
2-thiazolylthioglycolate, 44
8-quinolinol, effect on Fusarium wilt of tomato, 283
effect on toxicity of lycomarasmin, 284
8-quinolino sulfate, Rhizoctonia, 475
sulfamates, wheat leaf and stem rust, control and phytotoxicity, 496

Chemotherapeutants—*continued*
sulfanilic acid, wheat leaf and stem rust control and phytotoxicity, 497
thiazole, 44
2,3,5-triiodobenzoic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
2,4,6-trinitrobenzoic acid, wheat stem rust, 661
zineb, tobacco blue mold, 486
Chemotherapy, Dutch elm disease, 486
Fusarium wilt of tomato, effect upon, 137
oak wilt, 475
plant growth-regulators, 137
radiophosphorus inhibition of tobacco mosaic virus, 208
tobacco blue mold control with zineb and ferbam, 486
wheat rusts, 496, 659
Chenopodium album, tobacco-etch virus, local lesion host, 568
CHEO, PEN CHING, 78
CHEREWICK, W. J., 461, (697)
Cherry, disease control, mist and hydraulic spraying compared, 465
flower inoculum for virues, 479
fluoride concentrations, foliar, factors affecting, 587
green ring mottle virus, 481
quantitative method of assay on cucumber, 176
leaf spot, control, 109, 468
recurrent chlorotic ring pattern effect of aging on infectivity of isolates in inoculum from vegetable marrow and pumpkin, 325
ring spot virus, effect on growth of nursery trees, 480
Sclerontinia fructicola, and *S. laxa*, pathogenicity compared, 589
tatter leaf virus, infectivity and longevity, effect of temperature and age of source of inoculum on, 326
quantitative method of assay on cucumber, 176
trunk and crown rot, British Columbia, 402
virus complex, flower inoculum for isolations, 479
virus seed transmission, quick method, 467
yellows virus, infectivity of isolates, 325
quantitative method of assay on cucumber, 176
Chestnut blight, tannin factor in resistance, 32
Chick-pea, blight fungus, breeding for resistance, 580
CHILDS, JAMES F. L., 99, 101
China aster, effect of methyl bromide on, 376
CHINN, S. H. F., 701
Chloramphenicol, see under Antibiotics
Chloris gayana, 310
Chlorogenus myricae, 19
p-chlorophenyl p-chlorobenzene sulfure, see Insecticides, miticides, Ovotran
Chloromycetin, see under Antibiotics
Chloropicrin, see under Soil fumigants
CHRISTENSEN, J. J., 468, (479)
CHRISTIANSEN, D. W., (483)
CHRISTIE, J. R., 295
Chromatography, detection and assay of radioactive toxins *in vivo*, 237
Chrysanthemum, bacterial blight, dissemination and varietal susceptibility, 524
pathogen: *Erwinia chrysanthemi*, 526
symptoms, 522
methyl bromide injury, 489
stunt virus, 406
Marmor chrysanthemi, proposed name, 406
tomato aspermy virus on, 404
Cicer arietinum, see Chick-pea
CINNAMON, C. A., (439)
Cintractia spp., nomenclature, changes in, 183
Cirsium arvense, *Verticillium albo-atrum* on, 588
Citrus, concentric canker, 99
feeder roots, microorganisms isolated from, 395
Fomes applanatus associated with wood rot on, 99
Citrus—*continued*
Fusarium spp. on feeder roots, 395
gummosis, 101
paradisi, see Grapefruit
reticulata, see Tangerine
sinensis, see Orange
tristeza, evidence of in Florida, 51
grafting experiments, 51
wood rot of, 99
Cladosporium cucumerinum, cucumber breeding for resistance to, 257
cucumber varieties, new, resistant to, 215
effect on tissues of resistant and susceptible cucumber varieties, 481
fulvum, tomato varieties immune from, 109
spp. on soybean, 292
Clarkia elegans, *Phytophthora parasitica*, susceptibility to infection by, 290
Clavacin, see Antibiotics, Patulin
CLAYTON, E. E., 239, 468
Clitoria mariana, see Butterfly pea
Clover, alfalfa black stem on, 378
alsike, clover-root nematode on, 606
red clover vein-mosaic virus, 473
crimson, cucumber mosaic virus strains on, 336
for bioassay of wound-tumor virus, 387
Ladino, alfalfa mosaic strains on, 271
clover-root nematode on, 606
mosaic virus complex, 271
effect on yield, 273
red clover vein-mosaic virus, 473
yellow bean mosaic strains on, 271
red, bean yellow mosaic virus, 470
vein-mosaic, age of plant and method of inoculation, 473
incidence and host hange, 473
sweet, necrotic-lesion strain of yellow bean mosaic virus on, 46
Pythium spp., pathogenicity on seedlings, 474
white, clover-root nematodes on, 603
sweet, cucumber mosaic virus strains on, 336
red clover vein-mosaic virus, 473
wound-tumor virus, insect vectors, 9
yellow sweet, red clover vein-mosaic virus, 473
Coccomyces hiemalis, see Cherry leaf spot
control by actidione, 468
Cochliobolus sativus, inheritance of characters, 487
COCHRAN, L. C., 585, (691)
Colladonus geminatus, see Insects, under leafhoppers,
Colletotrichum destructivum, grass and legume isolates compared, 487
on brome grass, 486
gossypii, survival on cotton seeds in storage, 220
hibisci, kenaf varieties resistant to, 647
lagenarium, pH effect on lesions on cucumber leaves, 489
watermelon breeding for resistance to, 254
lindemutheanum, pressure effects on, 70
phomoides, perfect stage, 285
Common vetch, alfalfa black stem on, 378
Conifer, damping-off, seedlings, control, 446, 466
seedlings, disease complex etiology and control, 469
Conks, sterile of *Fomes igniarius*, 699
CONOVER, ROBERT A., 344
COOK, HAROLD T., 592
Cook, Melville Thurston, Biographical sketch, 591
COOLEY, J. S., (50)
COONS, GEORGE H., 297, 405
COOPER, D. C., (490)
COOPER, W. E., 289
CORBETT, M. K., 469
Corn, awl nematode, 296
charcoal rot, pycnidial stage of fungus on kernels, 608
cucumber mosaic virus strain on, 336
Gibberella stalk rot, loss of leaf area effect, 477

Corn—*continued*
Hormodendrum kernel rot, 386
injury, autogenus, 386
internal temperatures of root and stem compared with soil, 317
Pythium root rot, effect of aldehyde on virulence of pathogen, 289
spp. on seedlings, effect of temperature and moisture, 477
seedling blight control, 477
seedling root rot, 476
smut, gall development, effect of temperature on, 656
partial vacuum method inoculating seedlings, 654
stalk rot effect on yield, 479
sting nematode, 295, 435
stubby-root nematode, 296
Corticium galactinum, white pine needle blight studies, 468
praticola, as perfect stage of *Rhizoctonia solani*, 488
Corynebacterium flaccumfaciens, causing orange color in bean seed, 483
michiganense, tomato seed transmission, 473
sepedonicum, potato bags, ultra-violet, X-ray and nuclear radiation, effect of, on, 439
potato resistance to, 251
Cotton, *Ascochyta* blight, dusting for control, 292
gossypii on, 294
spp. on, 469
fiber properties and wilt resistance, new hybrids, 292
Fusarium wilt, metabolites production of vascular browning, 490
new resistant hybrids, 292
resistance, basis of, 489
nematode-wilt complex, soil fumigation for, 292
Rhizoctonia solani, dusting for control, 292
root-knot on Deltapine 15, 292
root rot, tests for control, 589
sting nematode, 434, 438
causing, loss of resistance to Fusarium wilt, 475
thrips, dusting for control, 292
Verticillium wilt, 585
breeding for resistance to, 489
varieties reaction to, 489
COURTNEY, W. D., (598)
Cowpea, alfalfa black stem on, 378
alfalfa yellow mosaic on, 39
Cercospora purple stain, seed, 477
cucumber mosaic virus strains on, 334
Fusarium wilt, races on, 291
necrotic-lesion strain of yellow bean mosaic virus on, 46
sting nematode, 435
COX, R. S., (419), 469
Criconema spp., see Nematodes, ring
Criconemoides spp., see Nematodes, ring
CRITTENDEN, H. W., 405
Cronartium quercuum, pycniospore stage on *Pinus taeda*, 488
strobilinum, pycniospore stage on *Pinus caribaea*, 488
CROSSAN, D. F., 469
Crown gall, organization of tumor cells in presence of naphthalene acetic acid, 204
Crucifer viruses, thermal inactivation and dilution end-point of strains, 545
Cucumber, anthracnose, effect of pH on development of lesions on leaves, 489
downy mildew, breeding for resistance to, 257
Fusarium wilt, 481
mosaic virus, breeding for resistance to, 256
powdery mildew, breeding for resistance to, 257
Rhizoctonia foliage blight on, 290
scab, breeding for resistance to, 257
effect of pathogen on tissues of resistant and susceptible varieties, 481
resistant varieties, new, 215
sour cherry tatter leaf virus isolates in, 326
Cucumber—*continued*
stone-fruit, green ring mottle virus isolates in, 325
latent virus transfer to, by cherry flower inoculum, 479
necrotic ringspot virus isolates in, 325
viruses, test plant for virus assay, 175
tomato aspermy virus on, 404
Cucumis sativus, see Cucumber
Cucurbits, disease resistance, breeding for, 254
inhibition of stone-fruit virus by diluents of, 328
stone-fruit virus isolates in, 324
Cultures, preservation of under oil, 407
CUMMINS, GEORGE B., (178)
CUNNINGHAM, H. S., 95
CURL, E. A., 61, (287), 469
Curled dock, clover-root nematode on, 606
Curvularia lunata, "fading-out" on turf grass, control, 109
spp. on soybean, 292
Cuscuta spp., bayberry-yellows virus, 15
Cyamopsis tetragonoloba, see Guar
Cylindrocladium scoparium, control on conifer seedlings, 469
Cycloheximide, see Antibiotics, actidione
Cynodon dactylon, 310
Cynosurus echinatus, 310

Dactylella sp., nematode capture by, 405
Dactylis glomerata, 309
Damping-off, conifer seedlings, control, 469
control by copper sulphate, 125
sugar beet complex, 300
temperatures of root and stem effect on, 317
tobacco seedlings, Venezuela, 125
DARLEY, ELLIS F., 469, (588)
DARLING, H. M., (476)
Dasyscycha willkommii, on larch, disease survey, 405
Datura spp., tomato bushy-stunt virus strains, test plant, for 485
stramonium, alfalfa yellow mosaic on, 39
cucumber mosaic virus strains on, 334
Daucus carota, bayberry-yellows virus on, 18
DAVIES, M. E., (109)
DAVIS, DAVID, (43), 137, 470
DAVIS, LILY H., (470), (585), 586
DEAN, JACK L., 290
DELP, CHARLES J., (475)
Dendrophoma obscurans, on strawberry, control, 471
DESJARDINS, P. R., 687
DEVAY, J. E., 470, (654)
DIACHUN, STEPHEN, 470
Diaporthe citri, shell bark relation to, 467
Diazouracil, effect on multiplication of TMV, 589
2,3, dichloro-1,4-naphthoquinone, see Fungicides, Phygon
2,4-dichlorophenoxyacetic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
dichloropropene-dichloropropane, see Soil fumigants, DD
DICKSON, JAMES G., 349, (473), (478), (527), (581)
DIENER, T. O., 586
3,5-dimethyltetrahydro-1,3,5,2-thiadiazine-2-thione, see Fungicides, Crag 974
DIMOCK, A. W., (522)
DIMOND, A. E., 43, (137), 195, 229, (281), 319, 470, (488), 619, 663
Dinitrocarprylphenyl crotonate, see Fungicides, Karathane
2,4 dinitrophenol, effect on plant respiration, 224
Diplocarpon earliana, strawberry, photographic infection scales for roguing susceptible seedlings, 345
Diplodia natalensis, on peanuts in market, 474
shell bark relation to, 467
zeae, effect on corn yield, 479
Disease development,
Gladiolus corms, effect of heat-curing upon, 141
effect of humidity upon, 142

Disease resistance, alfalfa to leaf spot, method of measurement, 651
avocado to Phytophthora root rot, 490
barley varietal crosses to loose smut races, 369
bean to TMV, acquired, 490
cabbage, Badger Market, new variety to yellows and mosaic, 650
to yellows, basis of, 489
celery, breeding for, 254
chick-pea to blight fungus, breeding, 580
cotton and cabbage vascular Fusaria effect of culture filtrates on, 489
to Fusarium wilt, 292, 489
to Verticillium wilt, breeding, 489
cucumber to scab, host cell reaction, 481
cucurbits, breeding for, 254
flax to rust, 624
varieties to Fusarium wilt races of pathogen, 495
identifying method, interspecific transfer, 468
kenaf to *Colletotrichum hibisci*, 647
multiple, in cotton, 292
in oats, breeding, 291
in sugar beets, breeding, 295
oats to crown rust, effect of temperature and age of plant, 484
Persea spp. to Phytophthora root rot, 490
plant growth-regulators inducing, 137
potato, nature of, 245
varieties, *Phytophthora infestans* races in Mexico, 454
sugar beet to curly-top virus strain, 588
varieties and inbreds resistant to virus yellows, 405
tobacco breeding for, 469
black shank field inoculation effectiveness for testing resistance, 289
tobacco, nature of, 243
to black shank altered by root-knot nematode, 483
to mosaic virus, 475
tomato, to *Cladosporium fulvum*, 109
Fusarium wilt resistance affected by metabolic inhibitors, 483
wheat breeding, rust, "composite variety," 467
rust differences in seedling and adult stage, 468
stem rust breeding, 472
linkage between resistance to race 15B and susceptibility to races 49 and 139, 472
to bunt, conditions affecting, 473
Dithane D 14, see Fungicides, Nabam
Dithane Z 78, see Fungicides, Zineb
Ditylenchus dipsaci, see under Nematodes
sp., culture of, for use in teaching and research, 105
Dolichodorus heterocephalus, see Nematode, awl
Dolichos lablab, alfalfa yellow mosaic on, 39
DOOLITTLE, S. P., 333, (404)
Dothiorella sp., apple, associated with cankered branches, 407
DRECHSLER, CHARLES, 405
Drosophila melanogaster, see under Insects
Dry rot, sprout inhibitors, effect of, on—in stored potatoes, 95
DUNEGAN, J. C., 405
DURBIN, RICHARD D. 470, 586
DURRELL, L. W., 60

EARHART, R. W., 290, 516
EDGERTON, C. W., 591
Eggplant, *Ascochyta* spp. on, 469
cucumber mosaic virus strains on, 334
potato virus Y on, 217
Verticillium albo-atrum isolates on, 466
EIDE, CARL J., (486)
EISENSTARK, A., (27)
Electrophoretic fractionation, tobacco ring spot virus, 687
mobility, tobacco ring spot virus, 690
tobacco mosaic virus, groups of strains, compared, 687
ELLIS, D. E., 290, (293), (469)
Elm, Dutch elm disease therapy of, 486
Elsinoe boucheae, spot anthracnose of *Bouchea*, 406
Elymus canadensis, *Ustilago striiformis* and *U. bullata* hybrids on, 548
spp., 201, 310
EMERSON, F. H., (109)
Empoasca fabae, *Solanum* spp., resistance to, 246
ENCISO, T., (472)
Endive, effect of air temperature and nitrogen supply on injury by olefinic peroxide fumigants, 588
Endoconidiophora fagacearum, see also *Chalara quercina* chemotherapy, 475
compatibility types A and B from naturally and artificially inoculated red oaks, 341
compatibility types in trees, 645
distribution in trees, 644
Drosophila melanogaster vector, 466
effect of temperature and humidity on longevity of spores, 476
fungus pads, 287
growth and development on C^{14}-labeled medium, 490
longevity in oak lumber and products, 485
Nitidulidae associated with, 466
fimbriata, differences in susceptibility of sweet potato varieties to, 78
Endothia parasitica, tannin factor in resistance to, 32
Endothlaspis spp., nomenclature, changes in, 183
Endria inimica, see Insects, under leafhoppers
ENGLISH, HARLEY, 586
Entyloma, nomenclature, changes in, 183
Enzyme action, control of in cell, 221
effect of quinones and ketones on, 226
β-glucosidase, Fusarium wilt infected tomatoes, 470
pectic, of *Botrytis cinerea* on potato tissue, effect, 340
of *Pythium debaryanum* on potato tissue, effect, 340
role in Fusarium wilt of tomato, 535
pectin methyl esterase, tomato Fusarium wilt, 472, 535
pectinase, *Chalara quercina*, produced in culture of, 447
pectinesterase, vascular browning, 490
polygalacturonase, tomato Fusarium wilt, role, 535
vascular browning, 490
polyphenol oxidase, *Sclerotinia fructicola*, relation to fungitoxicity, 483
Stemphylium sarcinaeforme, relation to fungitoxicity, 482
respiration, effect of toxins on, 221
tomato reaction and enzyme activity, 536
vivotoxins, 232
Eragrostis cilianensis, wheat striate mosaic virus on, 539
Ergot, loose smut associated with, 461
ERWIN, DONALD C., 586
Erwinia amylovora, greenhouse test for removing pear seedlings susceptible to infection by, 161
pear, inoculation of seedlings, 161
susceptibility tests, 405
Pyrus spp. crosses, effect upon, 161
atroseptica, black leg on potato, caused by, 92
chrysanthemi, bacterial blight of chrysanthemum, 526
physiological studies, 525
phytophthora, 92
spp., chrysanthemum, cross-inoculation tests, 525
effect of, on culture tissue, 92
cultural characters of, 526
Erysibe spp., nomenclature, changes in, 184
Erysiphe cichoracearum, culture on sunflower tumor tissue *in vitro*, 343
graminis, effect on respiration of wheat leaves, 223
environmental conditioning, effects on susceptibility of barley, 162
tritici, temperature effect on, on wheat seedling varieties in greenhouse, 290
polygoni, bean, control in laboratory, 477
effect on respiration of clover leaf, 223
pressure effects on, 70

Ethyl alcohol, pea, triple response to, 664
tomato Fusarium wilt, 663
Ethylene, auxin-ethylene balance, 232
effect on respiration of apples, 225
effect on respiration of lemon and pear, 222
epinasty in Fusarium wilt of tomato, 470, 664
pea, effect on, 664
production by *Fusarium oxysporum* f. *lycopersici*, 664
toxic action, mode of, 232
Ethyl mercuric p-toluene sulfonanilide, see Fungicides, Ceresan M
Ethyl mercury chloride, see Fungicides, Ceresan

False smut, on buffalo grass, etiology, 488
Favret, Ewald A., 471
Feazell, George D., 290
Feder, William A., 471
Feldmesser, Julius, (471), 471
Felix, E. L., 290
Fergus, Charles L., 103
Ferguson, John, (375), 471, 586, (593)
Ferric dimethyl dithiocarbamate, see Fungicides, ferbam
Festuca spp., 309, 310
Figs, Kadota, Alternaria rot control, 586
Cladosporium surface mold control, 586
Fir, Douglas, disease complex control, 469
Fischer, George W., 181, (200), 547
Fisher, H. H., (45), (407)
Flax, Fusarium wilt races, varietal reaction to, 493
rust, epidemiology in North Central States, 624
physiologic race survey from 1931, 625
races, genetic characters, 626
varieties resistant to, fungus races interaction, 625
Septoria linicola, dissemination, 468
Fletcher, D. G., 471
Flor, H. H., 624
Fluoride, concentrations in foliage, factors affecting, 587
Folsom, Donald, (108)
Fomes applanatus, concentric canker, associated with, 99
wood rot of citrus, associated with, 99
igniarius, aspen, white heartrot, 699
sterile conks on aspen, 699
Foster, H. H., 290
Fowler, Dorsey L., (277)
Fowler, Marvin E., 405, 406
French, Alex M., 513
Friedman, Bernard A., 108, 412
Froehlich, H. P., (477)
Fulton, Robert H., 471
Fulton, Robert W., 472
Fumigation, peroxide, olefinic bean leaf injury and protectants, 588
effect of air temperature and nitrogen supply on injury to herbaceous plants, 588
Fungi, effect of different pathogens on temperature of infected leaves, 679
mycorrhiza-like fungus in potatoes, 490
physiology, oxidizing and reducing agents effect on *Chalara quercina* growth in culture, 442
sclerotia, viability vs. colonization by soil organisms, 471
sterile conks, *Fomes igniarius*, 699
wood rotting in buildings, 20
Fungicidal action, nature of, 476
Fungicides, general, combined soil and foliage treatment, conifer seedling complex, 469
effect of fertilizers on effectiveness in soil, 590
liquid concentrates vs. dusts for onion smut control, 480
methods for evaluating effects on *Verticillium* in soil, 593
mixtures in control of apple scab and quince rust, 109
polyelectrolytes as spray conditioners, 489
sensitivity to, difference in single conidial isolates, 490
soybean autumn seed treatments, 476
spore germination and hyphal growth, differential action, 476
Fungicides, general—*continued*
testing of fungicides on apple seedlings in greenhouse, 109
tests for fungicidal solution strength for bulb dips, 598
uptake from dilute solution by fungus spores, 480
vapor action, mercury seed treatments, oats, 465
Fungicides, Aagrano 350, peanut seed treatment, 291
aconitic acid, fungicidal action, 476
Agrox, oat seedling blight, vapor effect, 465
aniline derivatives, fungicidal action, 476
Arasan, see also thiram
Arasan SF, soybean seed treatment, 476
biphenyl derivatives effect on apple fruit flavor, 385
effect on *Monilinia fructicola* on apples, 383
bis(p-Nitrophenol) carbonate, peach brown rot, 290
Bordeaux, actidione compared, cherry leaf spot, 468
apple black rot, 268
conifer seedling disease complex, 469
effect of continual use on black rot of apple, 268
effect of spraying muscadine grapes, 630
injury, effect on temperature of leaves, 678
oak leaf-blister, 472
Cadsol, 109
calcium polysulphides, root rot, cotton and alfalfa, tests, 589
Calocure, conifer needle injury, 469
captan (N-trichloromethylthio tetrahydrophthalimide), 58
and actidione mixture, wheat covered smut, 461
apple scab and quince rust, 109
bean halo blight, 407
blueberry blossom and twig blight, 481
oak leaf-blister, 472
peach brown rot, 407
peach canker, 109
peanut seed treatment, 291
rose blackspot, 406
strawberry gray mold, 482
strawberry leaf blight, 471
tomato damping-off, 291
C.C.C. 224, and 5400, peanut seed treatment, 291
Ceresan, (ethyl mercury chloride) peanut seed treatment, 291
shallot pink root, 294
test for solution strength, bulb dip, 602
Ceresan M (ethyl mercuric p-toluene sulfonanilide) oat seedling blight, 465
seed treatment, barley smut, 393
barley seedling blight, 392
oat, 290
oat, Helminthosporium blight, 391
oat smut, 393
wheat bunt, 393
wheat loose smut, 315
sodium nitrate effect on effects of, in soil, beet seedling necrosis, 590
test for solution strength, bulb dip, 598
Ceresan, New Improved (ethyl mercury phosphate) ferbam compared with, in control of corm-borne disease of gladiolus, 154
gladiolus corm-borne disease control, 154
Lysol compared with, in control of corm-borne disease of gladiolus, 154
root injury to gladiolus, 154
soybean seed treatment, 476
test for solution strength bulb dip, 602
tomato damping-off, Vancide 51 interaction, 291
chloranil (tetrachloro-para-benzoquinone) alfalfa black stem, 379
barley loose smut seed treatment, 313
gladiolus corms, 429
peanut seed treatment, 291
shallot pink root, 294
soybean seed treatment, 476

Fungicides—*continued*

chlorinated hydrocarbons, root rot, cotton and alfalfa, control tests, 589
o-chlorobiphenyl, effect on *Monilinia fructicola*, 383
Cop-O-Zink, oak leaf-blister control, 472
copper, impregnated paper wrap in peach rhizopus and brown rot, 407
strawberry leaf blight control, 471
Copper A, mist and hydraulic spraying, 465
copper oxide, conifer needle injury, 469
copper oxysulfate in fungiside-insecticide mixtures, roses, blackspot, 406
copper sulphate, damping-off in tobacco seedbeds, 125
tobacco seedlings, effect on growth, toxicity, 125
copper, tri-basic, polyelectrolytes as spray conditioners, 489
sulphate, oak leaf-blister, 472
zinc and DDT combination, cotton dusting, 292
Crag 341 (2-heptadecyl glyoxalidine acetate) apple scab and quince rust, 109
effect on fig, Cladosporium surface mold and Alternaria rot, 586
Crag 974 (3,5-dimethyltetrahydro-1,3,5,2-thiadiazine-2-thione) *Fusarium moniliforme*, 465
nematodes, 465
Penicillium sp., 465
Pythium spp., 465
Cuprocide, tomato damping-off, Vancide 51 interaction, 291
dimethyldithiocarbamic acid, sodium salts of and 2-mercaptobenzothiazole mixture, conifer damping-off soil treatment, 466
Dow-9-B, gladiolus corm injury, 429
Dowicide A, peach brown and rhizopus rots, 407
Elgetol (sodium salt of dinitro ortho cresol), Tag 331 compared, strawberry leaf blight, 472
ethylenebisthiuram sulfides, bean powdery mildew, 477
ferbam, (ferric dimethyl dithiocarbamate) apple, black rot, 268
apple scab and quince rust, 109
bean halo blight, 407
blueberry blossom and twig blight, 481
conifer seedling disease complex, 469
gladiolus corm-borne disease, 151
peach canker, 109
strawberry leaf blight, 471
tobacco blue mold, 486
tomato injury, 291
tomato, damping-off, 291
p-fluorobiphenyl, effect on *Monilinia fructicola*, 383
formaldehyde, conifer seedling disease complex, 469
damping-off, conifer seedlings, 469
root-rot, conifer seedlings, 469
tests for solution strength, bulb dip, 598
fungicide 406, see captan
heavy metals, fungicidal action, 476
2-heptadecyl-2-imidazaline, uptake from solution by fungus spores, 480
heterocyclic compounds, fungicidal action, 476
hydrocarbons, fungicidal action, 476
insecticide, dust mixtures, rose blackspot, 406
Karathane (dinitrocarprylphenyl crotonate) bean powdery mildew, 477
lime-sulfur, apple black rot, 268
injury, effect on temperature of leaves, 678
Lysol, gladiolus corm-borne diseases, 151
magnetic sulfur, apple black rot, 268
Manzate (manganese ethylene bisdithiocarbamate) apple black rot, 268
blueberry blossom and twig blight, 481
combined with insecticides, rose blackrot, and effect on plant, 406
conifer seedling disease complex, 469
fig, Cladosporium surface mold and Alternaria rot control by and phytotoxicity, 586
nitrate fertilizer effect on effects of in soil, 590
oak leaf-blister, 472
peach brown rot, 290
peach, effect of color on, 290
protectant against olefinic peroxide injury to beans, 588
Mathieson 275, peanut seed treatment, 291
2-mercaptobenzothiazole and dimethyldithiocarbamic acid mixture, conifer damping-off soil treatment, 466
Merculine, oak leaf-blister, 472
mercuric chloride, shallot pink root, 294
Mersolite 8 (phenyl mercuric acetate), test for solution strength, bulb dip, 598
Microgel, oak leaf-blister, 472
Monsanto 4367, 4370, oak wilt, 475
nabam and zinc sulphate, oak leaf-blister, 472
naphthoquinones, chromatographic separation of, 110
effect upon respiration, 226
effect upon sulfhydryl containing enzymes, 226
orthocide, see Captain, coined name, 58
Panogen (methyl mercuric dicyan diamide) oat seedling blight, 465
oat seed treatment, 290
test for solution strength, bulb dip, 598
Penn Salt NP770, peach brown rot, 290
phenols, fungicidal action, 476
N-phenylmercuriethylenediamine, apple scab and quince rust, 109
phenyl-mercury compounds, bluegrass, *Helminthosporium vagans*, 476
Phygon (2,3,dichloro-1,4-naphthoquinone) alfalfa black stem, 379
apple scab and quince rust, 109
blueberry blossom and twig blight, 481
peach canker, 109
shallot pink root, 294
Phygon XL, effect on fig, Cladosporium surface mold and Alternaria rot, 586
oak leaf-blister, 472
PMAS, conifer needle injury, 469
polyethyl biphenyl, effect on *Monilinia fructicola*, 383
Puratized, conifer needle injury, 469
shallot pink root, 294
quaternary ammoniums, fungicidal action, 476
quinones, fungicidal action, 476
naturally occurring, 226
Semesan, alfalfa black stem, 379
shallot pink root, 294
setrete, oat seedling blight, vapor effect, 465
Spergon, see chloranil
SR-406, captan, coined name, 58
sulfur, ferbam, insecticide mixture, rose blackspot and effect on plants, 406
magnetic "70," apple scab and quince rust, 109
wettable, bean leaf injury, 477
bean powdery mildew, 477
mist and hydraulic spraying, 465
peach brown rot, 290
with BHC and DDT, cotton dusting, 292
Tag 331 (phenyl mercury acetate), 109
strawberry leaf blight, 471
strawberry leaf injury, 472
Tersan, alfalfa black stem, 379
tetrachloroethylene, peach rhizopus rot fumigation, 407
tetramethylthiuram sulfides, bean powdery mildew, 477
thiocarbamates, fungicidal action, 476
thiram, (tetramethylthiuram disulfide) alfalfa black stem, 379
bean powdery mildew, 477
conifer damping-off soil treatments, 466
conifer seedling disease complex, 469
corn seedling blight, 477

Fungicides, thiram—*continued*
onion smut seed treatment, 596
peanut seed treatment, 291
pelleting conifer seed, 466
shallot pink root, 294
soybean seed, 189, 476
tomato damping-off, Fermate interaction, 291
Thiuram, peach canker, 109
toximycin as, 23
trichloroethylene, peach rhizopus rot fumigation, 407
undecylenic acid, oak wilt, 475
Vancide 51 (sodium salts of dimethyl dithiocarbamic acid and of 2-mercapbenzothiazole)
blueberry blossom and twig blight, 481
fig Cladosporium surface mold and Alternaria rot, 586
oak leaf-blister, 472
oak wilt, 475
peach brown rot, and plant injury, 290
tomato damping-off, Ceresan, New Improved, and Cuprocide interaction, 291
Vancide 51 ZW, soybean seed treatment, 476
Zinc Coposil, fig Cladosporium surface mold and Alternaria rot, 586
zinc sulphate, tri-basic copper combination, cotton dusting, 292
zineb, (zinc ethylenebisdithiocarbamate) conifer seedling disease complex, 469
effects of spraying compared with Bordeaux, muscadine grape, 631
fig Cladosporium surface mold and Alternaria rot, 586
oak leaf-blister, 472
protectant against olefinic peroxide injury to beans, 588
tobacco blue mold, 486
ziram (zinc dimethyl dithiocarbamate) blueberry blossom and twig blight, 481
fig Cladosporium surface mold and Alternaria rot, 586
Fungitoxicity, benzothiazole and related compounds to Fusarium wilt, 43
Sclerotinia,—of benzothiazole and related compounds to, 43
Stemphylium,—of benzothiazole and related compounds to, 43
Fungus sectoring, in *Fusarium lini*, 488
Fungus species, on peanuts in market, 474
Fungus spores, fungicides, uptake by, 480
Fusarium bulbigenum, 397
conglutinans, races 3 and 4, 465
races causing wilt of cowpea, 291
Elegans section, citrus feeder roots, 395
equisetti, citrus feeder roots, 395
lateritium f. *pini*, host range and geographical distribution, 475
lini, chromatic variants in, 488
Martiella section, citrus feeder roots, 395
moniliforme control, 465
survival on cotton seeds in storage, 220
oxysporum, 397
on soybean, 292
tobacco varieties resistant to, 240
toxic metabolites, 116, 490
f. *apii*, 254
f. *callistephi*, methyl bromide control, 376
f. *conglutinans*, cabbage resistance to, 649
races 1, 2, and 3, 280
f. *cubense*, soil moisture and type, effect on growth and survival, 499
corm lesions, 146
control, 151, 154, 376
heat-curing of corms infected by, 141, 425
"walling-off" by cork tissue, 430
wound periderm formation in corms related to, 425
f. *lini*, flax varieties reaction to races, 493
methods of inoculation, 491
pathogenic clones, selection and maintenance, 492
Fusarium—*continued*
races, pathogenicity, 493
soil type and temperature effect on virulence, 492
f. *lycopersici*, ammonium-N and nitrate-N levels in culture filtrate, 196
chemotherapeutic activity of benzothiazole and related compounds, 43
ethanol and ethylene production, 470, 664
β-glucosidase production of, 470
lycomarasmin, 116, 195
magnesium sulfate effect of, 466
nutritive sprays on host effect on, 466
osmotic properties of leaf parenchyma in diseased plants, 619
pectic enzymes, 472, 535
physiology, 195
polysaccharides in culture filtrates, 196
resistance affected by metabolic inhibitors, 483
sensitivity of single conidial isolates to fungicides, 490
toxin demonstrated by intergeneric grafts, 470
transpiration, losses and velocity, 619
vascular reaction of host in tomato to, 116
f. *melonis*, muskmelon resistance to infection by, 255
f. *niveum*, cucumber Fusarium cross-inoculations, 481
watermelon resistance to, 255
f. *pisi*, races 1 and 2, resistant pea variety, New Era, 473
f. *raphani*, 277
roseum, carnation clones identical with *F. roseum* f. *cerealis*, 485
cross-pathogenicity tests, 485
sambucinum, 95
semitectum, citrus feeder roots, 395
solani, effect on respiration of potato tubers, 222
var. *Martii*, 397
sp., on cucumber, 481
wound cork, effect of on penetration of, 95
spp., celery seedbed, control, 293
isolation from plant tissues, improved method, 700
tobacco seed-bed incidence, 125
vasinfectum, nematode complex on cotton, soil fumigation, 293
soil microorganisms growth in presence of, 482
Fusicoccum persicae, control, 109
Futrell, Maurice C., 290, 472

Gainor, G., 509
Gallicchio, Virginia, (478)
Galls, *Agrobacterium tumefaciens*, colonial variants, stability in, 510
cedar-apple rust in tissue culture, 360
organization and recovery of crown-gall cells in presence of α-naphthalene acetic acid, 204
tissue in gummosis of grapefruit, 101
tomato, golden nematode, cytology, 471
Garren, Kenneth H., (644)
Gastridium ventricosum, 310
Genetic factors, chick-pea resistance to blight fungus, 580
in *Cochliobolus sativus*, 487
color and mating type in *Helminthosporium sativum*, 487
disease resistance, method of identifying suited to interspecific transfer, 469
in flax to rust, 626
in wheat, resistance to yellow rust, 471
Genetics of microorganisms, *Glomerella cingulata*, linkage groups, 489
graminicolus smut fungi, in induced hybridization, 547
Melampsora lini, genes for virulence, 626
Ustilago striiformis hordei and *U. bullata* hybrids, inheritance of sorus and spore characters, 547
zeae, pathogenicity of biotypes, method to determine, 654
variability in sensitivity to fungicides, 490

Geranium, bacterial stem rot, 588
GERDEMANN, J. W., 603
Gibberella saubinettii, effect on respiration of potato slices, 222
zeae, corn stalk rot, effect of loss of leaf area, 477
effect on corn yield, 479
GIBLER, J. W., (468), 472
GIDDINGS, N. J., 587, (684)
GILMER, R. M., 108
GINOZA, W., 587
Gladiolus, bean virus 2 affecting, 111
bean yellow mosaic virus, 114
Botrytis neck rot, 167
cork formation, conditions favoring, temperature optimum, 426
corm-borne disease control, 151
corm lesion incidence, 146
corm mummifiaction, 430
corms, *Botrytis* on, 141, 146
disease control by heat-curing, 141
Fusarium oxysporum on, 141, 146
Penicillium spp. on, 149
Rhizoctonia solani on, 148
Rhizoglyphus rhizophagus, on, 150
Sclerotinia on, 148
wound periderm, effect of heat-curing upon, 141
cucumber mosaic virus affecting, 111
flower inoculum for virus isolations, 479
fungicide injury, 429
Fusarium basal rot, 146
yellows, heat treatment for control, 425
"walling-off" by cork tissue, 430
wound periderm formation related to, 425
histological studies of tissue reaction to Botrytis neck rot, 170
root injury, ethyl mercury phosphate, 154
Stemphylium leaf spot, *Pleospora* sp., conidial stage, 585
tobacco ringspot virus affecting, 112
virus complex, 114
Globe amaranth, cucumber mosaic virus strains on, 336
Glomerella cingulata, antibiotic assay, for, 23
camellia dieback and canker, 466
genetics of mutant characters, linkage groups, 489
muscadine grape, 629
glycines, 487
Glomerella phomoides Swank sp. nov., 285
Glucose-1-phosphate, effect on multiplication of TMV, 589
Glycine max, see Soybean
GNAGY, R. M., (587)
Gnomonia veneta, on white oak, weather related to occurence of, 103
Gomphrena globosa, see Globe amaranth
GOLD, A. H., (206), 458
GOLDEN, A. MORGAN, 406
GOODE, MONROE J., 472
Gossypium spp., Verticillium wilt, reaction to and breeding for resistance, 489
GOTHOSKAR, S. S., 472, (483), 535
GOTO, S., 472
GOTTLIEB, DAVID, (478)
GOULD, C. J., (598)
Grafting, citrus triteza studies, 51
transmission of phony disease virus, 692
GRAHAM, J. H., 189, 193, 577, 642
GRAHAM, S. O., 587
GRAHAM, T. W., 291, (291), 434, (475)
Gram, see Chick-pea
Gramicidin, see Antibiotics
Graminicolus smut fungi, induced hybridization, 547
GRANT, THEODORE J., 51
Grape, degeneration related to Pierce's disease, 611
Macrophoma ripe rot, 629
Melanconium leaf and stem fleck on, 347
muscadine, angular leaf spot, 629
effect of spraying on yields and disease incidence, 629
Grape—*continued*
rots: black and bitter, 629
shelling of berries, varietal variation, 632
Pierce's disease virus, insect vectors, 293, 612
yellow mosaic virus, 475
Grapefruit, actinomycete associated with gummosis of, 101
wood rot and canker associated with *Fomes applanatus*, 99
Grass, blue, control of diseases on varieties, 476
brome, *Colletotrichum destructivum* on, 486
brown patch, susceptibility of various lawn grasses to, 110
buffalo, false smut, etiology, 488
cereal yellow-dwarf virus, immune species, symptomless carriers, 310
Curvularia "fading-out," 109
head smut of, differentiation of races, 201
orchard, purple leafspot, effect of periods of high and low humidities on, 642
seed treatment, 487
striped smuts, host range and distribution, 527
GRAVES, CLINTON H., JR., 473
GREEN, G. J., 473
GREEN, MAURICE, 473
GREENLEAF, W. H., 564
GRIES, GEORGE A., (343)
GRIFFITH, R. B., 473
GRISWOLD, CHARLES L., (466)
GROGAN, R. G., 473
GROSSBARD, ERNA, 108
Growth regulators, see Plant growth regulators
Guar, alfalfa black stem on, 378
Guayule, charcoal rot, 633
GUBA, E. F., 109
Guignardia bidwellii f. *muscadinii*, 629
Gummosis, actinomycete, associated with on grapefruit, 101
GUNKEL, W. W., (480)
Gymnoconia peckiana, 360
Gymnosporangium juniperi-virginianae, mycelium on callus in tissue culture, 362
sporulation on galls in tissue culture, 362

HAASIS, F. A., (469)
HAFIZ, ABDUL, 580
HAGEDORN, D. J., 473
HALLER, MARK H., (406)
HALPIN, J. E., 474
HAMILTON, J. M., 109
HAMMACK, MARIAN G., 587
HANSON, E. W., (473), (474)
HARDING, R. B., (477)
HARE, W. W., 291, 474
HARRISON, A. L., 291
HART, HELEN, (485)
HASKETT, W. C., 474
HAYDEN, E. B., 474
HEIBERG, BARBARA C., 474
HEIM, JEAN M., 343
Heliconia spp., resistance to *Marasmius stenophyllus*, 479
Helicotylenchus spp., see Nematodes, spiral
Helixin B, see Antibiotics
Helminthosporium, inoculum preparation technique, 401
sativum, inheritance of characters, 487
spores in soil, method of estimating population, 478
wheat crown and root rot, 484
spp., control, 391
differential host reactions and conidial measurements, 516
vagans, bluegrass, control, 476
victoriae, 230
oat, breeding for resistance, 291
vapor action of mercury seed treatments, oats, 465
HEMERICK, G. A., (378)
HENDERSON, L., (468)
HENDRIX, J. W., 587, (643)

Henry, A. W., 461
Henry, Berch W., 81, 474
Henson, Lawrence, (470)
2-heptadecyl glyoxalidine acetate, see Fungicides, Crag 341
Hepting, George H., 475
Hess, G. E., (687)
Heterodera cruciferae, on Brussels sprouts, 260
rostochiensis, see also Nematode, Golden and tobacco cyst nematode, host preferences, 106
schachtii var. *trifolii*, see also Nematodes, clover-root life history, pathogenicity and host range, 603
sp., host preference, 106
Hewitt, Wm. B., 475
Hibiscus cannabinus, see Kenaf
Hickman, Donald, (486)
Hilborn, M. T., 475, (481)
Hildebrandt, A. C., (92), 475, (490)
Hoffman, P. F., 475, (490)
Holdeman, Q. L., (291), 291, (434), 475
Hollis, John P., 355
Hollyhock, *Ascochyta* spp. on, 469
Holmes, Francis O., 475
Holton, C. S., 219, 322, 398, (558)
Holtzmann, O. V., 587
Hooker, Arthur L., 476
Hoppe, Paul E., 386
Hopperburn, potato, resistance to, 252
Hordeum nodosum, 201
spp., 310
Hormodendrum corn kernel rot, 386
Horner, C. E., 587, 588
Horseradish mosaic virus, effect of air temperatures on, in *Nicotiana glutinosa*, 550
white rust, overwintering, 485
Horsfall, James G., 476, (482)
Hotson, Hugh Howison, 360, 659
Houston, Bryon R., (128), 271, (309), (458), 491
Howard, F. L., 109, 476, (486)
Hubert, Ernest E., 403, 637
Hughes, M. B., (50)
Humidity, Botrytis neck rot of gladiolus, relation to, 167
effect on longevity of spores of oak wilt pathogen, 476
effect on production and survival of sporangia of *Phytophthora infestans*, 507
gladiolus corms, effect on disease development on, 142
Hungerford, C. W., 519
Hutchins, Lee M., 691
Hydnaceae, wood rotting by 21
Hydrangea macrophylla, tomato ringspot virus on, 404
Hydrogen cyanide production by alfalfa crown rot pathogen, 581
Hydrogen-ion concentration, antibiotic production in soil and manures effect on, 108
Chalara quercina, effect on growth, 442
Colletotrichum lagenarium, effect on lesions on cucumber leaves, 489
Fusarium oxysporum f. *lycopersici* culture filtrates, 195
wheat leaf and stem rust, effect on chemotherapeutic action, 498
Hyre, R. A., 419

Indole acetic acid, see Plant growth regulators
Inheritance of characters, *Glomerella cingulata*, linkage groups, 489
graminicolus smut fungi, in induced hybridization, 547
Inheritance of resistance, barley to loose smut races, 369
cabbage to yellows and mosaic, 650
chick-pea to blight fungus, 586
flax to rust, 624
kenaf to *Colletotrichum hibisci*, 647
multiple, sugar beet, 297
oats to cold, 291
tobacco to mosaic virus, 475
wheat crosses to yellow rust, 471
Inheritance of susceptibility, to autogenous necrosis, cabbage, 415
Inoculum, flower tissue, cherry, virus complex, 479
flower tissue, gladiolus, cucumber mosaic virus, 479
leaf and flower compared, stonefruit viruses, 480
plant disease fungi, large quantity preparation, 401
Insecticides, BHC, cotton, 292
DDT, cotton, 292
lindane-fungicide mixtures, roses, effect on, 406
wheat stem rust, 661
lindane and DDT-fungicide mixtures, roses, effect on, 406
miticides, Aramite in fungicide-insecticide mixtures, roses, effect on, 406
Ovotran, fungicide-insecticide mixtures, roses, effect on, 406
Systox, *Ditylenchus dipsaci* feeding habits related to effect on, 471
Rhobditis sp. feeding habits related to effect of, 471
Insects, *Aceria tulipae*, wheat streak virus, survival at low temperatures, 484
wind dispersal, 485
Agallia constricta, New Jersey potato yellow-dwarf virus, 10
wound-tumor virus, 387
Agalliopsis novella, wound-tumor virus, 9, 387
Agrilus bilineatus associated with oak wilt, 61
aphids, apple-grain, (*Rhopalosiphum prunifoliae*) cereal yellow-dwarf virus, 309
cereal yellow-dwarf virus, 128, 312
corn (*Rhopalosiphum maidis*) cereal yellow-dwarf virus, 132
grass (*Mocrosiphum dirhodum*) cereal yellow-dwarf virus, 132
false cabbage (*Rhopalosiphum pseudobrassicae*) Brassica nigra virus, 542
greenbug, cereal yellow-dwarf virus, 309
green peach aphid (*Myzus persicae*), Brassica nigra virus, 209, 542
potato virus A strains, 479
potato virus Y, 217
tomato aspermy virus, 404
English grain (*Macrosiphum dirhodum*), cereal yellow-dwarf virus, 312
Macrosiphoniella sanborni, tomato aspermy virus, 404
Myzus solani, tomato aspermy virus, 404
pea aphid (*Illinoia pisi*), Wisconsin pea streak virus, 484
Rhopalosiphum rufomaculatum, tomato aspermy virus, 404
potato virus Y, mechanical inoculation vs. aphid inoculation, 469
western celery mosaic, 29
Buprestidae associated with oak wilt, 62
Cerambycidae associated with oak wilt, 61
Drosophila melanogaster, oak wilt, 466
Hessian fly, wheat, crown and foot rot relation to, 467
hopperburn, potato resistance to, 252
leafhopper, *Carneocephala flaviceps*, grape Pierce's disease virus, 293
Colladonus germinatus, peach yellow leaf roll virus transmission studies, 561
Endria inimica, wheat striate mosaic virus, 537
injection techniques, 388
Solanum spp. resistance to, 245
for bioassay of wound-tumor virus, 387
Nitidulidae, oak wilt associated with, 466
oak wilt associated with, 61, 69
Rhizoglyphus rhizophagus, gladiolus corms, 150
root mites on gladiolus corms, 150
Scolytidae associated with oak wilt, 61
Septoria linicola dissemination, 468
thrips, cotton dusting for control, 292
transovarian passage of New Jersey potato yellow-dwarf virus, 10
of wound-tumor virus, 9

Insects—*continued*
viruses, loss of tranmissibility by, 466
yellow-headed sharpshooter Pierce's disease, 612
Internal cork disease, sweet potato, 50
Ipomoea batatus, see Sweet potato
Isoelectric point, tobacco mosaic virus, groups of strains compared, 587
Isolation of fungi from woody tissue, instruments, 403
Isoleucine solutions effect on multiplication of TMV, 589
Itersonilia sp., parsnip, manner of invasion, 485
IVANOFF, S. S., 291

JEDLINSKI, HENRY, (467)
JEFFERS, W. F., (406)
Jelly end rot, potato, 467
JENKINS, W. R., (406)
JENSEN, D. D., 561
JEWELL, FREDERICK F., 476
JOHNSON, H. W., 476, (477)
JOHNSON, S. P., 363
JONES, E. D., 476
JONES, FRED REUEL, 651
JONES, L. S., (585)
Juniperus virginiana, callus tissue in culture, *Gymnosporangium* on, 360
Jute, charcoal rot, pycnidial stage of fungus on, 608

KAESBERG, PAUL, (482)
KEIL, H. L., 477
KEITT, G. W., (23), 59, (391), (478), (481)
KELLER, JOHN R., 406
KELMAN, ARTHUR, 291
Kenaf, *Colletotrichum hibisci*, resistance to, 647
KENDRICK, E. L., 477
KENDRICK, JAMES B., SR., (473)
KENDRICK, J. B., JR., 477, 588, (588)
KERNKAMP, M. F., 378, 477, (584)
Ketones, effect upon respiration, 226
unsaturated, naturally occurring, 226
KEVORKIAN, ARTHUR G., 406
KILPATRICK, R. A., 292, (476), 477
KING, J. F., (487)
KLOS, E. J., (481)
KNOWLES, P. F., (491)
KOEHLER, BENJAMIN, 477
KOMMEDAHL, THOR, 478
KONZAK, C. F., 369
KOPPE, R. K., (587)
KUNTZ, J. E., (441), (448)

LACKEY, C. F., 588
LAMBERT, E. B., (471)
LANGLEY, B. C., (291)
Larch, European, canker disease survey, 405
LARSON, R. H., (479), 596, (649)
Lathyrus odoratus, see Sweet pea
LEANDER, F. JOHNSON, (479)
LEBEAU, J. B., 478, 581
LEBEN, CURT, (23), 391, (465), 478
LEDINGHAM, R. J., 478, (701)
LEGATOR, MARVIN, 478
Legumes, Cercospora purple stain on seeds, 477
seed treatments, 487
LEHMAN, S. G., 292, 460
Lemon, shell bark, fungi and wound healing relation to, 467
Leptographium sp., distribution, and inoculation studies, 637
on pine, western white and lodgepole, 637
symptoms, histology and phytotoxin tests, 639
Leptothyrium pomi, 471
Lespedeza, clover root nematode on, 605
Rhizoctonia aerial blight, soil source of inoculum, 293
Lethum australiense, see also Viruses, 467
Lettuce, Romaine, effect of air temperature and nitrogen supply on injury by olefinic peroxide fumigants, 588
Light effect on lesion formation in potato virus Y on *Physalis floridana*, 2
effect on reaction of Kubanka wheat to races of *Puccinia graminis tritici*, 481
effect on susceptibility of oat hybrids to stem rust, race 7, 473
effect on thiouracil inhibition of TMV, 555
effect on thiouracil up-take by tobacco leaves, 556
LINFORD, M. B., (603)
LINN, M. B., (466), (485)
LIVINGSTON, J. E., 496
LOCKWOOD, J. L., 478
LOEGERING, WILLIAM Q., 479
Loganberry, root rot, *Phytophthora* sp., *Pythium* sp., and nematode associated with, 588
Lolium multiflorum, 310
Lonicera hispidula, apple flyspeck host, 586
Lotus spp., *Stemphylium loti* on, 579
LOWNSBERY, B. F., 106
LUCAS, G. B., (289), (483), (701)
LUKE, HERBERT H., 479
Lupine, *Ascochyta* spp. on, 294
Cercospora purple stain, seed, 477
LUTTRELL, E. S., 347, 629
Lycium spp., potato virus A strains on 479
Lycomarasmin, characteristics of, 231
chelation with metals, 233, 282, 320
complexes, iron and copper relative stability of, 284
decomposition to non-toxic substance I, 319
decomposition rate *in vitro*, 320
effects on transpiration, compared with *Fusarium oxysporum* f. *lycopersici*, 116
ferro-lycomarasmin, decomposition compared with, 320
inhibition by copper ion, 282
inhibition by iron chelating agent, 281
inhibitors as chemotherapeutants, 283
iron complex, 116
non-competitive inhibitor, 233
physiology of production in cultures of *Fusarium oxysporum* f. *lycopersici*, 195
potentiation by iron ion, 281
production rate estimates *in vitro*, 320
8-quinolinol, effect on, 283
role in Fusarium wilt of tomato, 284
strepogenin, inhibited by, 233
temperature and pH effect on life of, 320
toxicity, 116, 199, 281
Lycopersicon esculentum, see Tomato
peruvianum, 109
root-knot nematode resistance, 290
pimpinellifolium, potato virus A strains on, 479
spp., buckeye rot, susceptibility to, 290

MACDONALD, H. A., (487)
MACLACHLAN, D. S., 479
Macrophomina phaseoli, as synonym for *Botryodiploida phaseoli*, 610
on peanuts in market, 474
pycnidial stage, 608
Macrosiphum spp., cereal yellow-dwarf virus vector, 132
Magnamycin, see Antibiotics
Malas baccata, *Venturia inaequalis*, races 2 and 3 on, 484
Maleic hydrazide, increase in size of pustules of wheat leaf rust, 497
MANDELL, J. D., 27
Manganese ethylene bisdithiocarbamate, see **Fungicides**, Manzate
MANKIN, C. J., (587)
MARAMOROSCH, KARL, (387)
Marasmius stenophyllus, sheath rot, abaca, 479
Marigold, tissue culture, effect of bacteria on, 92
Marmor chrysanthemi, see Chrysanthemum stunt virus
cucumeris, muskmelon, breeding for resistance, 256
medicaginis H. var. **flavovarians** N. var., 38
upsilon, see Viruses, potato Y

MARSHALL, BARTON H., JR., 425
MARTIN, J. P., (465)
MARTIN, W. J., 292, (292), 432
MAUGHAN, F. B., (477)
MAYHEW, D. J., (587)
McBETH, C. W., 264
McCALLAN, S. E. A., D1, (480)
McFADDEN, LORNE A., (522)
McINTOSH, D. L., 402
McKEEN, W. E., 588
McWHORTER, FRANK P., 479, (479)
MEINERS, JACK P., 200
Melampsora helioscopiae, effect on respiration of *Euphorbia peplus* leaves, 223
lini, 360
genetics of pathogenicity, interaction of races and flax varieties, 626
physiologic specialization of races, 624
survey of pathogenicity and epidemiology in North Central States since 1931, 624
Melanconium fuligeneum, grape, leaf and stem fleck on, 347
muscadine grape, 629
Melanin, amino acid stimulation of, in *Streptomyces*, 357
ascorbic acid in relation to, 361
formation in galls of cedar apple rust, 361
production correlated with growth of *Streptomyces*, 358
Melilotus alba, see Clover, white sweet
Meloidogyne arenaria, on pepper varieties, 474
hapla, on pepper varieties, 474
incognita, bean, 171
ovicidal and therapeutic value of various compounds, 485
potassium nutrients of host, effect upon development of, 171
sweet potato resistance to, 290
tomato, 171, 290
var. *acrita*, 292, 474
spp., see also Nematodes, root-knot, 260, 292
cotton, soil fumigation, 293
Fusarium wilt complex on cotton, 293
MENZIES, J. D., 684
Metabolism, effect of toxins on, 221
polyphenol oxidase system in cedar-aple rust fungus, 361
Methionine, effect on toxins, 233
Methocel, in seed treatment, 476
Methods, see Techniques
Methyl bromide, see Soil fumigants
Methylene blue, wheat stem rust, 661
Methyl mercuric dicyan diamide, see Fungicides, Panogen
MICHAELSON, MERLE E., 479
Microflora, on tobacco roots, diseased, 590
Microorganisms, culture under oil seal, 407
Microthyriella rubi, apple flyspeck, 470
ecology and host range, apple, 586
imperfect stage of, 470
MIDDLETON, JOHN T., (588), 588
MILBRATH, J. A., 479
MILLER, J. B., (489)
MILLER, JULIAN H., 292
MILLER, LAWRENCE P., 480
MILLER, PATRICK M., 480
MILLER, R. L., (461)
MILLER, V. L., (598)
Millet, sting nematode, 295
MILLIKAN, D. F., 480
MILLS, W. R., 454, (456)
MILSTREY, R. A., 406
Mineral element deficiency, see also Nutrition, potassium nutrition of host effect upon development of root-knot nematode, 171
Mint, rust, 178
wild species susceptible to races of mint rust, 178
MISHRA, J. N., 338
MITCHELL, J. W., (407), 480
Monilinia fructicola, biphenyl derivatives toxic to, 385
control with fungicides, peach, 290, 407
sensitivity of single conidial isolates to fungicides, 490
MOON, H. H., (405)
MOORE, M. B., (401)
MOORHEAD, ELLEN L., 73
Muhlenbergia asperifolia, *Tilletia asperifolia* on, spore germination, 219
MUNGER, H. M., 254
MUNNECKE, DONALD E., 375, 588
MURAKISHI, H., (643)
MURPHY, D. R., (109)
MURPHY, H. C., (474)
MURPHY, M. M., (629)
MURPHY, R. P., (487)
Musa spp., *Marasmius stenophyllus* on, 479
Mushroom-spawn nematode, temperature-time gradients in control, 404
Muskmelon, breeding for disease resistance, 255
fruit rots, *Phytophthora* sp. and other fungi causing, 293
weather effects on incidence of fruit rots, 293
Mustard, Brassica nigra virus on species, 543
Mycorrhiza, potato tubers and stems, mycorrhiza-like fungus, 490
Mycosphaerella angulata, muscadine grape, 629
fagariae, strawberry, photographic infection scales for roguing susceptibile seedlings, 345
rabiei, chick-pea breeding for resistance to, 580
Myrica carolinensis, bayberry-yellows virus on, 18
Myrothecium spp. in soil, inhibitory to damping-off of peppers, 586
Myzus persicae, see Insects, aphids, green peach

α-Naphthaleneacetic acid, effect of potato leafroll on response of tomato to, 109
effect on root development by tumor tissue of periwinkle, 204
Fusarium wilt of tomato, effect on, 137
metabolism of host, effect on, 139
methyl ester of, effect of, on Fusarium dry rot in potatoes, 95
1-4 naphthoquinones, see also Fungicides, chromatographic separation of, 110
β-naphthoxyacetic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect on, 139
Narcissus pseudonarcissus, Ditylenchus-induced leaf "spikkels," cytology and structure, 471
NARVAEZ, I., (472)
Nasturtium mosaic virus, Brazilian, cross-protection tests of strains, 307
distribution of, in inoculated plants, 304
Lycopersicum virus 3 relation to, 306
strains, 307
NATTI, JOHN J., 109
NEAL, D. C., 292
NELSON, PAUL E., 589
NELSON, R. R., (477), 584
Nematocides, 485
assaying techniques, 264
DD, 264
screening, 264
Nematodes, associated with killed sclerotia of *Verticillium*, 594
awl (*Dolichodorus heterocephalus*) control in celery seedbeds, 293
Brussels sprouts, *Heterodera cruciferae* on, 260
capture by, *Arthrobotrys* and *Dactylella* spp., 405
citrus, soil fumigation control, 466
clover-root (*Heterodera schachtii* var. *trifolii*) cyst and larvae measurements, 603
life history and host range, 604
control, Crag 974, 465
crop rotation for control and detection, 260
culture for teaching and research, 105

Nematodes—*continued*

cyst-forming, clover-root, 603
dagger (*Xiphinema americanum*), incidence and host range, 296
detection by fumigation, 260
diagnosis by analysis of mineral elements of plant parts, 260
Ditylenchus dipsaci, Narcissus, leaf "spikkels," cytology and structure, 471
Systox, feeding habits related to action by, 471
water screen testing, 264
sp., laboratory culture of, 105
temperature-time gradients in control in mushroom-spawn, 404
ectoparasitic, incidence and host range, 295
field diagnosis of infestations, 259
field testing, 265
fungi complex control in celery seedbeds, 293
Fusarium wilt complex on cotton, 292
golden (*Heterodera rostochiensis*) tomato, cytological effects of, 471
Helicotylenchus sp. on cotton, 292
laboratory preservation and permanent mounts, 262
meadow (*Pratylenchus*) control, 438
sting-compared, 437
sugarcane, associated with diseased roots of, 289
mushroom-spawn, moisture and heat, effect on control, 404
temperature-time gradients in control, 404
nomenclature problems, 262
Panagrellus redivivus, contact nematicidal value of various compounds, 485
Paratylenchus spp., 297
loganberry root rot, associated with, 588
pine root rot, in relation to, 81
pine seedlings, root rot control, 474
Rhabditis sp., Systox, feeding habits related to action, by, 471
ring, *Criconema* spp. and *Crinonemoides* spp., 297
on peanuts, 297
Procriconema spp., 297
root-knot (*Meloidogyne* spp.), 260
African violet, 406
bean, lima, 171
clean fallow for control on soybeans, 405
complex, control in celery seedbeds, 293
on cotton, soil fumigation, 293
on pepper varieties, 474
on sweet potato, 290
on tomato, 171, 290, 465
ovicidal and therapeutic value of various compounds, 485
potassium nutrients of host, effect upon development of, 171
soil injection screening, 265
tobacco, effect on resistance to black shank, 483
Rotylenchulus reniformis on cotton, 292
soil fumigation effect on population, 474
soil injection screening, 265
soil sampling methods of detection, 261
spiral, on Amaryllis, 297
staining plant tissues for detection, 261
sting (*Belonolaimus gracilis*), body measurements, 436
control, 291, 293, 438
cotton, loss of resistance to Fusarium wilt, 475
cotton wilt complex, 260
incidence and host range, 295, 435
meadow, compared, 437
pathogenicity tests, 436
population trends, effect of various plants in greenhouse pot culture, 291
stubby-root, incidence and host range, 296
sugar-beet, 260
techniques for detecting and investigating, 259
temporary laboratory mounts, 262

Nematodes—*continued*

tobacco cyst, host preference, 106
Trichodorus sp. control in celery seedbeds, 293
Tylenchorynchus sp. on cotton, 292
sugarcane, associated with diseased roots of, 289
Neurospora crassa, 359
NEWHALL, A. G., (254), 480
NEWSOM, L. D., 292
Nicandra physaloides, Phytophthora parasitica, susceptibility, 290
potato virus A strains on, 479
potato virus Y on, 217
NICHOLS, CARL W., 555
Nicotiana glutinosa, horseradish mosaic virus air temperature studies, 550
nasturtium mosaic virus, 306
tomato bushy-stunt virus strains on, 485
rustica, cyst nematode, 106
spp., cabbage black ring and virus A, air temperature studies, 553
cucumber mosaic virus, resistance to, 472
cucumber mosaic virus strains on, 334
disease resistance, 243
potato virus Y on, 217
turnip virus 1, air and soil temperature studies, 550
turnip virus 1, effect of nutrition on multiplication in, 669
tabacum, see Tobacco
NIEDERHAUSER, JOHN S., (454), 456, 480
NIENSTAEDT, HANS, 32
Nitidulidae, 466
Nitrogen, see under Nutrition
Nomenclature, see also Taxonomy
Botryodiploidia phaseoli, Macrophomina phaseoli as synonym, 610
Marmor chrysanthemi, stunt virus, 406
nematodes, 262
species concept for classification of smut fungi, 181
Ustilago striiformis, 530
Non-parasitic disease, cabbage, autogenous necrosis, 415
potato jelly end rot, 467
NORTON, DON C., 633
Nothopanax guilfoylei, see Panax
Nutrient solution, effect on plant regulators, effect on rooting of *Prunus mahaleb*, 587
Nutrition, calcium, effect on tomato and potato susceptibility to *Alternaria solani*, 476
concentration of solutions, effect on growth of *Nicotiana* spp., multiplication of turnip virus 1, 671
magnesium, effect on potato and tomato susceptibility to *Alternaria solani*, 476
nitrogen, effect on multiplication of turnip virus 1 and plant growth, *Nicotiana* spp., 669
effect on potato and tomato susceptibility to *Alternaria solani*, 476
phosphorus and potassium levels in relation to multiplication of TMV, 488
Pseudomonas solanacearum response to sources and osmotic pressure of, 291
supply effect on injury by olefinic peroxide fumigants on herbaceous plants, 588
tomato, TMV concentration in, 477
phosphorus, effect multiplication of turnip virus 1 and plant growth, *Nicotiana* spp., 670
effect on potato and tomato susceptibility to *Alternaria solani*, 476
potassium, bean, effect on root-knot nematode, 171
effect on multiplication of turnip virus 1 and plant growth, *Nicotiana* spp., 670
effect on potato and tomato susceptibility to *Alternaria solani*, 476
of host, effect upon development of root-knot nematode, 171
potato, effect on susceptibility to *Alternaria solani*, 476

Nutrition—*continued*
salicylic aldehyde, effect on virulence of Pythium root rot pathogen, 289
tobacco in relation to multiplication of TMV, 488
mosaic virus effect of nitrate, phosphate and potassium levels on growth of host tissue and virus titer, 475
tomato, effect on susceptibility to *Alternaria solani*, 476
turnip virus 1, effect on multiplication in *Nicotiana* spp., 669
Nutrition of microorganisms, amino acids, *Phytophthora infestans* races A and B response to, 514
ammonium salts, *Phytophthora infestans* races A and B responses, 515
asparagine, *Phytophthora infestans* races A and B responses to, 514
biotin effect on growth of *Chalara quercina*, 443
calcium, inhibition of *Sclerotium rolfsii*, 363
carbon, effect on soil microflora, 365
sources for growth of *Charlara quercina*, 445
Chalara quercina, 441
inositol effect on growth of *Chalara quercina*, 443
nitrogen, amino acids as sources for *Venturia inaequalis*, 481
effect on soil microflora, 365
salts effect on isolates of *Sclerotium rolfsii*, 363
sources for growth of *Chalara quercina*, 444
sources for growth of *Venturia inaequalis*, 481
Pseudomonas solanacearum, nitrogen, effect of osmotic pressure, 291
nitrogen, response to different sources of, 291
Pythium root rot of corn, effect of aldehyde on virulence of pathogen, 289
raw sugar, growth stimulant, *Phytophthora infestans* races A and B, 515
succinic acid, *Phytophthora infestans* races A and B responses, 515
thiamine effect on growth of *Chalara quercina*, 443
vitamins and purines, growth of *Chalara quercina*, 443
yeast extract, growth stimulant, *Phytophthora infestans* races A and B, 515
zinc and silicon or gelatin requirement in rust germ tube development, 483

Oak, laurel, dagger nematode, 296
leaf-blister control, 472
rust, pycniospore stage on pines, 488
white, anthracnose, 103
wilt, carbon sources for growth of pathogen in culture, 445
chemotherapy, 475
compatibility types of fungus in trees, 341, 655
control, 406
distribution of the fungus in trees, 644
effect of temperature and humidity on longevity of spores of pathogen, 476
fungus, 61
fungus pads, 287
host responses, 448
inoculum potential vs. season, 469
insects associated with, 69
insect transmission studies, 466
longevity of pathogen in lumber and wood products, 485
nitrogen sources for growth of pathogen in culture, 444
pathogen on C^{14}-labeled medium, 490
radioactive rubidium in water movement, infected trees, 450
spore germination of pathogen vs. season, 469
status, 406
transpiration of host, 450
tyloses in host tissue, 451
vitamin effect on growth of pathogen in culture, 443
Oats, breeding for resistance to cold, 291
cereal yellow-dwarf virus, 309
aphid vectors, 309, 312
epiphytotic, conditions favoring, 310
crown rust, breeding for resistance, 291
effect of temperature and age of plants on reaction of varieties to, 484
effect of air temperature and nitrogen supply on injury by olefinic peroxide fumigants, 588
Helminthosporium blight, seed treatment, 391
spp., reactions of varieties to isolates of, 517
rust, race 4 of pathogen, rapid spread in Argentina, 487
stem, biotypes within race 7 of pathogen, 472
effect of temperature and light, 472
seedling blight, vapor action of mercury seed treatments, 465
seed treatments, 290, 391, 393
Siberian mosaic virus and wheat striate mosaic compared, 539
smut, control, 393
covered, inoculating method, 697
Victoria blight, breeding for resistance to, 291
wheat striate mosaic virus, 539
yellow-dwarf virus, 130
effect on yield, 135
Ogawa, Joseph M., 589
Okimoto, Marion, (465)
Okra, *Ascochyta gossypii* on, 294
spp., on, 469
Olive, Russian, Phytophthora canker, 468
Onion, "Alternaria blotch," 411
Alternaria spp., pathogenicity on bulbs and leaves, 410
storage, 411
bayberry-yellows virus, 18
blast, *Botrytis* spp., incitant, 483
brown blotch pathogens, 409
leaf spotting or blast, *Botrytis* spp., incitants, 483
purple blotch pathogens, 409
smut, control, thiram seed treatment, 596
liquid concentrates vs. dusts for control, 480
Orange, wood rot and canker associated with *Fomes applanatus*, 99
O'Reilly, H. J., 481
Orellana, Rodrigo G., 125
Orthocide, see Fungicides, captan coined name, 58
Oswald, John W., 128, (271), 309, (458), (473)
Oteifa, Bakir A., 171, (407)
Owen, John H., 481

Palm, Washington, Phytophthora trunk rot, 469
Palmiter, D. H., 109
Panax, ringspot virus, symptoms and transmission, 643
Panicum capillare, 310
Pantothenic acid, 221
Papavizas, George C., 481
Parasites, facultative, and obligate, nature of plant reactions to, 353
relation to host metabolism, 221, 227
Parker, K. G., 481
Parsnip, leafspot and canker, manner of invasion by pathogen, 485
Pasteur effect, effect of antimycin A on, 227
loss of, in wheat infected with powdery mildew, 224
Pate, J. B., 647
Patulin, see Antibiotics
Pea, alfalfa black stem on, 378
clover-root nematode on, 606
cucumber mosaic virus strains on, 334
disease resistant variety, New Era, 473
Fusarium, metabolites production of vascular browning, 490
root rot control, soil fumigation, 590
Wisconsin streak virus, pea aphid vector, 484
Peach, antibiotics, effect on, 405
Armillaria mellea, wilt-inducing pigment from, on, 486
bacterial spot spring canker, 465

Peach—*continued*
brown rot control, 290, 406
canker control, 109
cherry yellows transmission to plum scions, 108
leaf drop control, 405
mosaic virus, spread related to age of infection, 585
necrotic ringspot transmission to plum scions, 108
phony disease virus in tops of trees, 692
rhizopus rot control, 406
Sclerotinia fructicola, and *S. laxa*, pathogenicity compared, 589
western X virus, ninhydrin-reacting substance in leaves, 586
physiological changes induced by, 481
yellow leaf roll virus, leafhopper transmission studies, 561
Peanut, alfalfa black stem on, 378
collar rot, heat canker phase, 571
fungi associated with in market, 474
heat canker, occurrence and symptoms, 571
soil temperature and sky condition effect, 572
nematodes on, 295, 297
Sclerotium rolfsii, pathogenicity of isolates on, 289
seed treatment, 291
Pear, fireblight, age of seedlings, effect on resistance to, 161
crosses resistant to, 156
disease rating, effect of temperature on, 160
inoculation method, seedlings, 156
on seedlings, 405
resistance, growth stage, 157
terminal shoot growth cessation, effect on resistance to, 161
streptomycin, effect on Bartlett seedlings, 405
Pecan, dagger nematode, 296
Pelargonium hortorum, see Geranium
PELLETIER, E. NEIL, 481
PELLETIER, R. L., 481
Penicillin, see Antibiotics
Penicillium digitatum, effect on respiration of lemon, 222
patulum, antibiotic production in soil and manures, 108
sp. control, 465
isolate from soil antagonistic to fungi and bacteria, 479
spp., in soil, inhibitory to damping-off of peppers, 586
on peanuts in market, 474
on soybean, 292
Pepper, *Ascochyta* spp. on, 469
cucumber mosaic virus, alfalfa mosaic virus complex on, 333
damping-off, inhibition by soil organisms, 586
effect of methyl bromide on, 376
nematodes, varieties resistant to, 474
Phytophthora blight, 469
potato virus Y on, 217
tabasco, tobacco-etch virus symptoms on, 565
tobacco-etch virus, multiplication and translocation in, 567
tomato aspermy virus on, 404
Verticillium albo-atrum isolates on, 466
Peppermint rust, method of evaluating severity and control, 587
oil gland destruction and leaf drop, 587
Verticillium albo-atrum, pathogenicity of isolates on, 588
Periwinkle, crown-gall tumor cell organization and recovery in presence of α-naphthalene acetic acid, 204
tissue culture, effect of bacteria on, 92
Peronospora effusa, pressure effects on, 70
manshurica, race 4, 292, 460
schachtii, sugar beet breeding for resistance, 300
tabacina, 239
control, 486, 616
distribution, geographical, 616
overwintering, 616
spore formation and germination, 701
spore showers, 616
PERRY, V. G., 293
Persea spp., Phytophthora root rot resistance, 490
PERSON, L. H., (290), 293, 701
Pestalozzia spp., in soil, inhibitory to damping-off of peppers, 586
PETERSON, E. A., (461)
PETERSON, L. C., (469)
Petunia, cucumber mosaic virus strains on, 336
hybrida, bayberry-yellows virus on, 18
potato virus Y on, 217
tobacco necrosis virus strain on roots, adaptation, 590
tomato aspermy virus on, 404
pH, see Hydrogen-ion concentration
Phalaris spp., 310
Phaseolus spp., see Bean
Phenyl mercury acetate, see Fungicides, Tag 331 and Mersolite 8
Phoma betae, sugar beet seedlings black rot, 300
Phomopsis sojae on soybean, 292
Phony disease, see under Peach
Phyllosticta spp. on soybean, 292
Phymatotrichum omnivorum, cotton and alfalfa root rot control tests, 589
Physalis angulata, tomato aspermy virus on, 404
floridana, potato virus Y, 1, 217
quantitative virus assay, 6
spp., *Phytophthora parasitica*, susceptibility to infection by, 290
tobacco-etch virus on, 568
Physalospora obtusa, apple rot in Maryland, 407
effect of continual use of fungicides, 268
Physiologic specialization, flax rust pathogen races, 624
Fusarium congultinans races 3 and 4, 465
races on cowpea, 291
races 3 and 4, 465
Peronospora manshurica race 4, 292, 460
Puccinia graminis tritici races on Kubanka wheat, 481
menthae, races, 178
Tilletia caries, races, pathogenicity, 398
T. foetida races in mixture, host passage survival studies, 558
Ustilago bullata, 200
nuda races, 369, 407
Venturia inaequalis, races 2 and 3, 484
Physoderma spp., nomenclature, changes in, 183
Phytophago destructor, see Insects, Hessian fly
Phytophthora cactorum, olive, Russian, 468
sweet cherry, 402
capsici, pepper, 469
summer squash, 469
cinnamomi control, 376, 465
root rot of avocado and *Persea* spp., 490
citrophthora, methyl bromide control, 376
cryptogea, alfalfa root rot, 586
fragariae, strawberry red stele soil fumigation and fungicide tests for control, 590
infestans, antibiotics effect on, 463
effect on respiration of potato tubers, 222
phaseoli, growth and viability in culture compared, 423
potato resistant varieties reaction to, 455
races, 480
A and B physiologic differences, 513
in Mexico, 454
survival in mixture, 486
Solanum spp. in Mexico, 456
sporangia production and survival, 505
virulent race of, on resistant tomatoes, 344
parasitica, tobacco breeding for resistance to, 239
toxin production in tobacco and tomato sap, 294
var. *nicotianae*, field inoculation effectiveness for testing resistance, 289
root-knot nematode effect on varietal resistance to, 483
var. *terrestris*, *Clarkia elegans* susceptibility to, 290
immune and resistant plant species, 290
phaseoli, effect of temperature, moisture and medium on growth and viability in culture, 419

Phytophthora phaseoli—*continued*
germination of sporangia and zoospores, 422
homothallism in, 423
infestans compared, 423
production of sporangia, 420
sp., loganberry root rot, associated with, 588
muskmelon fruit rots, 293
palm trunk rot, 470
tobacco black shank relation to, 290
PIERSON, C. F., (215), 481
Pigeon pea, alfalfa black stem on, 378
Pine, lodgepole, pole blight, 637
pitch canker, host range and geographical distribution, 475
pole blight, distribution and inoculation studies, 637
phytotoxin tests, 640
symptoms and histology, 639
root rot seedling control, 81, 474
seedling, nematodes associated with, 474
seedling damping-off, 466
white, blister rust, distribution, effect of microclimate, 487
disease complex control, 469
needle blight, *Corticium galactinum* studies, 468
western, pole blight, 637
Pisum sativum, see Pea
PITTS, RALPH, (519)
PLAKIDAS, A. G., 293, (466)
Plant growth regulators, in chemotherapy, 137
indole acetic acid, effect on Fusarium wilt of tomato, 137
effect on metabolism of host, 139
potato virus Y studies, 469
indolebutyric acid, effect on rooting of *Prunus mahaleb*, nutrient pretreatment effect on effect of, 587
maleic hydrazide, size of wheat rust pustules, 497
α-naphthaleneacetic acid, effect on Fusarium wilt of tomato, 137
effect on metabolism of host, 139
effect of potato leafroll on response of tomato to, 109
effect on root development by tumor tissue of periwinkle, 204
methyl ester of, effect on Fusarium dry rot of potato, 95
β-naphthoxyacetic acid, effect on Fusarium wilt of tomato, 137
effect on metabolism of host, 139
pantothenic acid, 221
Pleospora sp., on gladiolus, 585
Plum, Japanese, cherry yellows, transmission to, 108
necrotic ringspot, transmission to, 108
latent necrotic ringspot, quantitative method of assay on cucumber, 176
myrobalan, necrotic ringspot and cherry yellows, transmission to *Prunus salicina*, 108
phony disease virus in tops of trees, 691
Sclerotinia, fructicola and *S. laxa*, pathogenicity compared, 589
Poa spp., 309
POLLEY, DOROTHY, 598
Polycystis spp., nomenclature, changes in, 183
Polyelectrolytes as conditioners for fixed copper sprays, 489
Polymixin, see Antibiotics
Polyporaceae, wood rotting by, 21
Polyscias guilfoylei, see Panax
POPP, W., 482, 697
Populus tremuloides, see Aspen
Poria laevigata, yellow birch canker and decay incitant, 487
Potato, bacterial wilt, southern, 245
blackleg, 245
black scurf, *Rhizoctonia solani*, in India, 645
Botrytis cinerea, effect of maturity and water content on tubers on susceptibility to, 338, 339
charcoal rot, pycnidial stage of fungus on, 608
curly-top virus strain, 587, 684
disease resistance, 245
Potato—*continued*
diseases, multiple resistance, cultivated and wild species, 246
early blight, 245
effect of pectic enzymes of *Botrytis* and *Pythium* on, 340
Fusarium dry rot, 95
golden and tobacco cyst nematode host preferences, 106
green dwarf caused by strain of curly-top virus, 684
hopperburn, resistance to, 252
insect resistance, wild species, 245
internal brown spot associated with infection by *Verticillium albo-atrum*, 108
jelly end rot, 467
late blight, 245
antibiotics effect on, 463
races of pathogen, 480
resistant varieties reaction to races of in Mexico, 455
sporangia production and survival, 505
survival of races of pathogen in mixture, 486
leaf roll, 109, 245
mosaic virus, alfalfa and yellow bean strains on, 271
mycorrhiza-like fungus in tissue cultures, 490
nutrition effect on susceptibility to *Alternaria solani*, 476
"pink eye," associated with infection by *Verticillium albo-atrum*, 108
Pythium debaryanum, effect of sprouting, pre-heating and water content on susceptibility to, 339
ring rot, 245
on burlap bags, ultra-violet, X-ray and nuclear radiation effect of, on, 440
scab, common, 245
storage behavior when infected with *Verticillium albo-atrum*, 108
Synchytrium endobioticum on wild species in Mexico, 480
tissue culture, effect of bacteria on, 92
tobacco cyst nematode, 106
Verticillium albo-atrum, 108, 245
virus A, 245
strains, 479
strains, *Myzus persicae* vector, 479
virus X, 245
method for identifying immune seedlings, 487
virus Y, 1, 217
aphid transmission, 217
host range in Hawaii, 217
mechanical inoculation vs. aphid inoculation and field reaction, 469
strains of, 5
wart, resistance to, 251
yellow dwarf virus resistance to, 249
POUND, GLENN S., 277, 415, 482, (488), 550, (649), 669
Powdery mildew, barley, environmental conditioning, effects on susceptibility to, 162
cucumber breeding for resistance to, 257
muskmelon, breeding for resistance to, 255
tissue succulence related to susceptibility of barley, 162
POWELL, DWIGHT, (383), (465), 482
POWERS, H. R., JR., (483)
Pratylenchus, see under Nematodes
PRESTON, WILLIAM H., JR., (480)
PRICE, W. C., (73), (509)
Procriconena spp., see Nematodes, ring
Propylene oxide on soil, 108
Protomyces spp. nomenclature, changes in, 183
Prune, dwarf virus, infectivity and longevity of inoculum, 326
fluoride concentrations, foliar, factors affecting, 587
Prunus mahaleb, growth regulators and nutrient pretreatment effect on rooting, 587
salicina, see Plum, Japanese
Pseudomonas caryophylli, cause of wilting, carnation, 587
virulence, retention in culture, 406
fluorescens, *Verticillium albo-atrum* associated with, on potatoes, 108

Pseudomonas—*continued*
glycinea, field and laboratory tests on persistence, soybean, 189
inoculum dilution and soybean reaction, 468
soil and debris, persistence in, 191
lapsa and *P. syringae*, physiological reactions compared, 413
maculicola, broccoli bacterial spot, 583
phaseolicola, control, streptomycin sulfate, 407
solanacearum, nitrogen, response to different sources of in culture, 291
sp., blueberry stem canker incitant, 589
spp., serological tests with, 412
tabaci, field and laboratory tests on persistence, soybean, 189
soil and debris, persistence in, 191
Pseudoperonospora cubensis, muskmelon, breeding for resistance, 255
Pseudopeziza medicaginis, alfalfa resistance, rapid method of measurement of, 651
pure culture maintenance and inoculation procedure, alfalfa, 651
Pseudoplea briosiana, perfect stage of *Stemphylium botryosum*, 477, 584
Puccinia antirrhini, pressure effects on, 70
coronata, oat breeding for resistance, 291
var. *avenae*, germ tube development, zinc and silicon or gelatin requirement, 483
oat varieties, effect of temperature and age of plant on reaction of varieties, to, 484
glumarum, wheat crosses, races on, 471
graminis var. *avenae*, race 4 spread in Argentina, 487
race 7, 472
tritici, breeding for resistance, "composite variety" of wheat, 467
effect of chemotherapeutants on, 496, 661
races, 472, 485
races, Kubanka wheat, effect of temperature and light on reaction to, 481
helianthi, pressure effects on, 70
malvacearum, effect on respiration of *Althea rosea* leaves, 223
menthae, control, 180
epiphytology, 178
physiologic specialization in, 178
rubigo-vera tritici, control by chemotherapeutants, 496
Pyrus spp., fireblight, crosses resistant to, 156
Pythium arrhenomanes control, 465
penicillium antagonistic to, 479
debaryanum, potato, effect of pectic enzymes on, 340
graminicola, barley varieties resistant to, 474
irregulare, conifer damping-off control, 466
sp., loganberry root rot, associated with, 588
spp., celery seedbed, control, 293
corn seed decay and root rot, 476
effect of temperature and moisture on pathogenicity, corn seedlings, 477
pathogenicity on alfalfa, 474
pathogenicity on sweet clover, 474
sugar beet seedlings black rot, 300
ultimum, control, 376, 590
temperature related to damping-off by, 318
Quercus spp., see Oak
Quince Rust, control by fungicide mixtures, 109

Raabe, Robert D., 482
Radioactive elements, P^{32} effect on tobacco mosaic virus, 206
P^{32} tobacco plant injury, 207
Radioactive pathogens, 236
Radioactive tracers, C^{14}-labeled sucrose in oak wilt fungus study, 490
Ce^{144}, S^{35} and C^{14} in study of uptake of toxicants by fungus spores, 480
P^{32} tomato Fusarium wilt transpiration studies, 621
rubidium, oak wilt, 448
Radioisotopes, carbon^{14}, effect on microorganisms, 237
for tagging toxins, 236
detection of microbial toxins, 236
tritium, effect on microorganisms, 237
for tagging toxins, 236
Radish, cabbage yellows on, 277
Fusarium oxysporum f. *conglutinans*, 280
Fusarium wilt, cabbage yellows compared, 277
control, 280
races 3 and 4 of pathogen, 465
resistant lines of Scarlet Globe, 280
temperature effect, 277
varietal reaction to, 278
virus 1 group, aphid-transmissable, 545
Ram, C. S. Venkata, 482, (488)
Ramsey, G. B., (474), (583)
Ramularia spp., nomenclature, changes in, 183
Rape, Brassica nigra virus on varieties, 543
Raphanus sativus, see Radish
Rappaport, Irving, 589
Raski, D. J., 259
Rawlins, T. E., (89), (206), (589)
Rawlins, W. A., (350), (480)
Ray, B. Roger, (486)
Raychaudhuri, S. P., 15
Respiration of pathogen in relation to temperature of host, 679
relation of temperature in diseased, injured and healthy leaves, 679
toxins effect on, in plant tissue, 221
western X virus infected peach vs. healthy, 481
Rhamnus californica, apple flyspeck host, 586
Rheum rhaponticum, *Phytophthora parasitica* susceptibility, 290
Rhizoctonia, aerial blight of Lespedeza, soil source of inoculum, 293
ethyl mercury phosphate, control by in gladiolus corms, 154
foliage blight, cucumber, 290
Lysol and ferbam, control by, in gladiolus corms, 5
solani, bean seeds, 483
conifer damping-off control, 466
cotton dusting for control, 292
flax seedling blight, *Corticium praticola* as perfect stage, 488
gladiolus corm lesions, 149
inhibition by soil organisms, 586
lettuce, control, 475
methyl bromide control, 376
on potato in India, 645
potato stems, control, 475
tobacco seedlings, damping-off control, 125
tomato control, 475
turf brown patch, factors influencing, 484
sp., sugar beet seedlings black rot, 300
spp., celery, control in seedbeds, 293
web-blight symptoms on beans, 290
Rhizoglyphus rhizophagus, gladiolus corms, 150
Rhizopus nigricans, fungicide control on peach, 407
on soybean, 292
survival on cotton seeds in storage, 220
spp., on peanuts in market, 474
tritici, effect on respiration of sweet potato root, 222
Rhopalosiphum spp., see also Insects, aphids, cereal yellow-dwarf virus vectors, 132, 309, 542
Rhubarb, ringspot-like virus, 590
Rice, Robert V., 482
Rich, A. E., (109)
Rich, Saul, (476), 482
Richards, M. C., 109
Rieman, G. H., (490)
Riker, A. J., (92), (441), (448), (475), (487)
Rimocidin, see Antibiotics
Rodenhiser, H. A., 558
Root rot on pine seedlings, control by soil fumigation, 81

Rose bengal, in isolation of Fusarium spp. from plant tissues, 700
Rose, black spot, fungicide-insecticide mixture for, 406
rosette virus, 218
Ross, A. Frank, 1
Roth, Elmer R., (475)
Rotylenchulus reniformis, see Nematodes
Rowell, J. G., 654
Rubus spp., apple flyspeck, 586
Rust, bean, pressure effects on, 70
flax, epidemiology in North Central States, 624
physiologic race survey from 1931, 625
races, genetic characters, 626
varieties resistant to, fungus races interaction, 625
fungi, pressure effects on, 70
germ tube development, zinc and silicon or gelatin requirement, 483
mint, method of evaluating severity and control, 587
oak, pycniospore stage on pines, 488
oat crown, effect of temperature and age of plant on varietal reaction to, 484
on gall tissue of *Juniperus virginiana* in culture, 360
peppermint, oil gland destruction and leaf drop, 587
physiology, 360
snapdragon, pressure effects on, 70
sugar beet resistant varieties, 300
sunflower, pressure effects on, 70
wheat black stem, races of pathogen manner of infection of resistant varieties, 485
breeding for resistance, "composite variety," 467
leaf, distribution in Oklahoma, 294
leaf and stem, control by, and phytotoxicity of chemotherapeutants, 496
races, 294
stem, breeding for resistance, 472
infection type, Kubanka, 481
races, pathogenicity on Kenya varieties, 474
races, resistance, 472
seedling and adult changes in resistance, 468
spore shower, origin and extent, 471
white pine blister, distribution, effect of microclimate, 487
yellow, races of, on wheat crosses, 471
Rye, *Helminthosporium* spp., reaction to isolates of, 517
yellow-dwarf virus, 131

Saccharine, wheat stem rust, 661
Saintpaulia ionantha, root-knot nematode, 406
Sakimura, K., 217
Salicylic aldehyde, effect on virulence of Pythium root rot pathogen, 289
Sallans, B. J., (701)
Sambucus glauca, apple flyspeck, 586
Sasser, J. N., 483
Scab, cucumber, breeding for resistance to, 215, 257
Scheffer, R. P., 116, (472), 483, (535)
Schexnayder, C. A., (289)
Schlegel, David E., 89, 206, 589
Schnathorst, William C., 483
Schneider, Henry, (51)
Schroeder, H. W., 401
Schuster, M. L., 483
Sclerospora sp., on sugarcane, 289
Sclerotinia fructicola, effect of fungicides on spore germination and hyphal growth, 476
polyphenol oxidase relation to fungitoxicity, 483
S. laxa, pathogenicity compared on various hosts, 589
gladoli, corm lesions, 148
laxa, 589
sclerotiorum, bean, effect of storage of sclerotia on viability, 520
bean infection by perfect stage, 520
bean, occurrence and source of infection, 519
soil organisms on, 471
spp., fungitoxicity of sodium and potassium salts of 2-benzothiazolythioglycolate to, 43
Sclerotinia fructicola—*continued*
methyl bromide control, 376
Sclerotium bataticola, effect of temperature and moisture on growth, 634
linear growth rates, 633
delphinii, soil organisms on, 471
rolfsii, calcium inhibition of, 363
disintegration in alfalfa meal-soil mixture, 366
nitrite, toxic effect, 366
pathogenicity of isolates on peanuts, 289
soil microflora antagonism toward, 366
sweet potato circular spot, 432
spp., tobacco seed-bed incidence, 125
Scolytidae, see Insects
Scott, C. Emlen, (218)
Seed treatment, see also under Fungicides
barley loose smut control, 313
grasses and legumes, 487
mercury fungicides, vapor action of, 465
oat, 290
onion smut, thiram, 596
peanut, 291
pelleting, conifer damping-off, 466
tomato, damping-off, 291
wheat loose smut, 315
Segall, R. H., 483
Senseney, C. A., (687)
Septoria, barley leaf blotch, 473
apii-graveolentis, celery resistance to, 254
linicola, dissemination, 468
tritici, 294
Serological test for tobacco mosaic virus, 77
rabbit antisera, TMV strains, antigenic relationships, 589
with *Pseudomonas* spp., 412
Shallots, pink root control, 294
Shands, R. G., (472)
Sharp, E. L., 483
Shaw, Charles Gardner, (181)
Shaw, R. H., (317)
Shay, J. R., (156), 483
Sherbakoff, C. D., 395
Shurtleff, M. C., 110, 484
Siang, W. N., (219)
Siegel, Albert, 589, (589)
Silberschmidt, Karl, 304
Sill, W. H., Jr., 484
Silverborg, S. B., 20, 699
Simmonds, P. M., (478), 484, (701)
Simons, John N., 29
Simons, M. D., 484
Sitanion hystrix, 310
Skiles, R. L., 409
Skotland, C. B., 484
Slykhuis, John T., 484, 537
Smith, D. H., (474)
Smith, Floyd F., (404), 406
Smith, F. G., (483)
Smith, M. A., 583
Smith, W. L., Jr., (50), 406
Smut, barley, 393
covered, inoculation method, 697
ergot associated with, 461
fungi, nomenclature, system of classification for, 181
grass head, differentiation of races, 200
striped, host range and distribution, 527
specialized varieties and physiologic races of pathogen, 528
symptoms and pathological histology, 527
oats, control, 393
covered, inoculation method, 697
onion, control, thiram seed treatment, 596
wheat, control, 315, 461
loose, distribution in Oklahoma, 294
races of pathogen, 681

Snapdragon, cucumber mosaic virus strains on, 336
rust, pressure effects on, 70
Snow-mold, see Alfalfa, crown rot
SNYDER, WILLIAM C., (470), (586)
Sodium salts of dimethyl dithiocarbamic acid and of 2-mercaptobenzothiazole, see Fungicides, Vancide 51
Sodium salt of dinitro ortho cresol, see Fungicides, Elgetol
Soil, antibiotic production in, 108
Soil composition, injury from methyl bromide effected by, 489
Soil fumigants, general, citrus nematode, 466
fungi-nematode complex in celery seedbeds, 293
orange, effect on growth and yield, 465
pine root rot, 81
strawberry red stele control tests, 590
Verticillium control and methods of evaluating effects, 593
Soil fumigants,
allyl bromide, 81
CBP-55 (chlorobromopropene), 81, 293, 593
root rot, pea and bean, 590
chloropicrin, 81, 593
conifer seedling disease complex, root-rot and damping-off, 469
tobacco, effect on growth and *Pythium ultimum* infection, 590
DD (dichloropropene-dichloropropane), 81, 293, 466
nematode detection, 260
sting nematode control, 291
sweet potato circular spot, 433
dichlorobutene, 81, 293
Dowfume W-40, sting nematode, 438
EDB (ethylene dibromide), 81, 293, 466
effect on nematode populations, 474
Fusarium wilt-nematode complex, cotton, 293
nematode detection, 260
pine seedling root rot control, 81, 474
sting nematode control, 291
sweet potato circular spot, 433
formaldehyde, root rot control, pea and bean, 590
Larvacide, see chloropicrin
methyl bromide, 81, 293
effect on various plant species, 376
injury, carnation and chrysanthemum, 489
injury, effect of soil composition and other treatments upon, 489
various fungi, 376
Soil fumigation, apparatus for, 81, 590
Soil microflora, antagonism toward *Sclerotium rolfsii*, 366
associated with *Verticillium* sclerotia, 594
effect of carbon and nitrogen on, 365
effect of organic matter on, 367
Fusarium vasinfectum growth in presence of, 482
non-pathogenic organisms for reinfestation of sterilized soil, 586
on sclerotia, 471
Soil moisture, effect on growth and survival of *Fusarium oxysporum* f. *cubense*, 499
Soil temperature, effect of air temperatures and sky condition on, 573
effect on cabbage virus A in *Nicotiana* spp., 550
effect on heat canker of peanuts, 573
effect on turnip virus 1 strains in *Nicotiana* spp., 482
related to Botrytis neck rot of gladiolus, 167
root and stem, internal temperatures compared, 317
Soil treatments, conifer damping-off, 466
effect on growth of tobacco and strawberry, 590
soybean meal in wheat root rot control, 701
Soil type, *Fusarium oxysporum* f. *lini*, effect on virulence, 493
relation to moisture in growth and survival of *Fusarium oxysporum* f. *cubense*, 500
Soja max, see Soybean
Solanum demissum, late blight, races of pathogen on, 480
Phytophthora infestans races in Mexico, 454
melongena, see Eggplant
Solanum—*continued*
nodiflorum, potato virus Y on, 217
spp., *Heterodera* sp. on, 106
Phytophthora infestans on, 456
parasitica, resistance to infection by, 290
Synchytrium endobioticum on, in Mexico, 480
tobacco-etch virus on, 568
virus, resistance, 245
Sorghum spp., 310
Sorosporium spp., nomenclature, changes in, 183
SOWELL, GROVER, JR., 485
Soybean, *Alternaria* spp. on, 293
Ascochyta spp. on, 469
bacterial blight, inoculum dilution and host relation, 468
persistence of pathogen, 191
bacterial pustule, field and laboratory tests on persistence of pathogen, 189
wildfire, effect of presence of, 191
Cercospora kikuchii on, 292
purple stain, seed, 477
downy mildew race 4 of pathogen, 292, 460
fungi associated with seeds and pods, 292
necrotic-lesion strain of yellow bean mosaic virus on, 46
root-knot nematode, clean fallow for control, 405
seed treatment, autumn, 476
sting nematode, 295, 435
wildfire, bacterial pustule, effect of presence of, 191
field and laboratory tests on persistence of pathogen, 189
SPANGELO, L. P. S., 345
Sphacelotheca spp., nomenclature, changes in, 183
SPILKER, OREN W., 485
Spinach, clover-root nematode on, 606
cucumber mosaic virus strains on, 334
downy mildew, pressure effects on, 70
effect of air temperature and nitrogen supply on injury by olefinic peroxide fumigants, 588
tomato aspermy virus on, 404
Spinacia oleracea, see Spinach
Sporisorium spp., nomenclature, changes in, 183
Sporotricum sp., in bean seeds, 483
Spot anthracnose, Bouchea in Cuba, 406
SPRAGUE, V. G., (642)
SPROSTON, THOMAS, JR., 110
Sprout inhibitors, Fusarium dry rot of potatoes, 95
Squash, disease resistant hybrids, 257
summer, Phytophthora blight, 469
SR-406, captan, coined name, 58
STAGE-SMITH, R., 589
Stagonospora maculata, effect of periods of high and low humidities on development, orchardgrass, 642
STAHMANN, MARK A., (473), (482), (535)
STAKMAN, E. C., (470), (471), (474)
STALEY, JOHN M., (341)
STARR, C. H., 439
STEERE, RUSSELL L., 485
Stemphylium botryosum, alfalfa leaf spot, 477
conidial stage of *Pseudoplea briosiana* on alfalfa, 584
onion brown blotch, 411
loti sp. nov., birdsfoot trefoil, symptoms, 577
cardinal temperatures, pathogenicity and overwintering, 579
host range and cross-inoculation studies, 579
sarcinaeforme, polyphenol oxidase relation to fungitoxicity, 482
sp. fungitoxicity of sodium and potassium salts of 2-benzothiazolylthioglycolate to, 43
in bean seeds, 483
STESSEL, G. J., 23, (61), 65
STEVENSON, F. J., 245
STEWART, R. N., (406)
Stizolbium deeringianum, see Bean, velvet
Stone fruit, green ring mottle virus, 326, 329
latent virus complex, cherry flower inoculum, 479

Stone fruit—*continued*
necrotic ringspot virus, 325, 329
viruses in cucurbit hosts, effect of extracts on infectivity, 328
infectivity and longevity in cucurbit hosts, 324
quantitative method of assay on cucumber, 175
STONER, WARREN N., 293, 611
STOVER, R. H., 499, 700
Strawberry, gray mold control, 482
leaf blight control, 471
leaf scorch, photographic infection scales for roguing susceptible seedlings, 345
leaf spot, photographic infection scales for roguing susceptible seedlings, 345
red stele, soil fumigation and fungicide tests, for control, 590
sting nematode, 295, 435
STREETS, R. B., 589
Strepogenin, lycomarasmin inhibition of, 233
Streptomyces lavendulae, 359
scabies, 355
potato resistance to, 250
sp., antimycin A (A-102) produced by new isolate of, 478
spp., amino acid effect on melanin in, 357
melanin production and growth in, 355
toxin formation, 356
venezuelae, synthesis of chloramphenicol by, 478
Streptomycin, see Antibiotics
STROUBE, WILLIAM H., 293
STRUBLE, F. BEN, (290)
Sugar beet, breeding for disease resistance, 297, 301
breeding techniques, 301
black root, causal organisms and resistance, 300
crop: size, geographical distribution and economic importance of, 297
curly-top virus strain, nature of injury, 588
on potato, synonym green dwarf, 684
resistance to, 298, 588
virus yellows relationship, 587
downy mildew, non-bolting resistant varieties, 300
leaf spot, resistant varieties and hybrids from inbreds, 299
seed production enterprise, 298
varieties, combined disease resistance, 300
virus yellows, 301
virus yellows, curly-top relationship, 587
varieties, resistant, 302, 405
Sugarcane, growth-retarding disease in Louisiana, 289
nematodes, associated with diseased roots of, 289
Pythium root rot, salicylic aldehyde, effect on virulence of pathogen, 289
Sclerospora sp. on, 289
Sugars, carnation bacterial wilt studies, 587
Sulfa compounds, wheat rust, control and phytotoxicity, 497, 661
Sunflower, rust, pressure effects on, 70
tumor tissue *in vitro*, culture of powdery mildew on, 343
Sun-hemp, charcoal rot, pycnidial stage of fungus on, 608
SWAEBLY, MARY ANN, 485
SWANK, GEORGE, JR., 285, (293)
Sweet pea, cucumber mosaic virus strains on, 336
Sweet potato, black rot, differences in susceptibility of varieties to, 78
internal cork disease, varietal reaction to, 290
nematode, root-knot, resistance to, 290
Sclerotium rolfsii, circular spot, 432
sting nematode, 435
storage temperature, relation to internal cork development, 50
Sweet william, carnation Fusarium wilt on, 465
SYLVESTER, EDWARD S., (29), 209, 541
Symphoricarpos albus, *Phytophthora parasitica*, susceptibility to infection by, 290
Verticillium albo-atrum on, 588
Synchytrium endobioticum, potato resistance to, 251
wild potatoes in Mexico, 480
Syngonium auritum, black cane rot, 586
SZKOLNIK, MICHAEL, (109)

Tagetes signata, bayberry-yellows virus, 18
TAKESHITA, R. M., 485
TAMMEN, JAMES, 485
Tangerine, wood rot and canker associated with *Fomes applanatus*, 99
Taphrina coerulescens, oak, control, 472
TAPKE, V. F., 162, 407
TARJAN, A. C., 485, 486
Taxonomy, see also Nomenclature, *Erwinia chrysanthemi*, 525
Pseudoplea briosiana, 584
Stemphylium botryosum, 584
TAYLOR, GORDON S., 486
TAYLOR, JACK, 268
Techniques, alfalfa resistance to leaf spot, rapid method of measurement, classification of plants, 651
apple seedlings in greenhouse, testing of fungicides, 109
autoradiography of histological sections with labeled toxins, 237
bacteriophage in differentiating types of *Xanthomonas pruni*, 27
barley loose smut viability prolonged by storage temperatures, 407
chromatographic analysis of extracts of plants infected with radioactive pathogens, 237
chromatographic separation of 1-4 naphthoquinones, 110
culturing obligate parasites on excised tissue *in vitro*, 343
equipment, fungicide-insecticide dusting, seedling diseases and insect pests, 480
soil fumigation, 590
evaluating effects of soil fumigants on *Verticillium*, 593
field inoculation testing tobacco for black shank resistance, 289
freezing, causing breaking of TMV particles, 482
greenhouse test for susceptibility of pear seedlings to fireblight, 158
heat-ultra method for purification of TMV for spectrophotometric assay, 89
identifying potato seedlings immune from virus X, 487
inoculation, barley and oats seed with smut spores, 697
Fusarium oxysporum f. *lini*, 491
pear seedlings with *Erwinia amylovora*, 156
inoculum dilution in testing for resistance, soybean to bacterial blight, 468
flower and leaf compared, stonefruit viruses, 480
plant disease fungi, large quantity preparation, 401
insect injection, 388
isolation of fungi from woody tissue, instruments, 403
medium for tissue culture, fungi, 360
nematocides, assay, 264
nematodes, detection in the field, 259
diagnosis by analysis of mineral elements of plant parts, 260
laboratory culture for teaching and research, 105
laboratory preservation, 262
soil sampling for detection, 261
staining plant tissues for detection, 261
temporary and permanent mounts, preparation of, 262
paint spray gun, mass inoculation with potato virus X, 487
partial vacuum method of inoculating seedlings, *Ustilago zeae*, 654
pathogenicity of biotypes of *Ustilago zeae*, method to determine, 654
preservation of cultures under oil, 407
proving pathogenicity of vivotoxins, 229
pure culture maintenance and inoculation procedure, *Pseudopeziza medicaginis*, 651
radio carbon C^{14} in medium for oak wilt fungus, 490
radioisotopes, selection for toxin assay, 236

Techniques—*continued*
radio phosphorus in Czapek's medium, 478
rose bengal and streptomycin in isolation of *Fusarium* spp., 700
rust, mint, evaluating severity and control, 587
scale model to estimate reduced water flow in Fusarium wilt, 488
serological tests, rabbit anisera, TMV strains antigenic relationships, 489
sheep blood for tobacco mosaic virus, 77
spores in soil, population estimation, *Helminthosporium sativum*, 478
spraying, mist and hydraulic compared, 465
staining vascular elements, 117
strawberry, photographic selection scales, roguing seedlings susceptible to leaf spot and leaf scorch, 345
sugar beet breeding for resistance, 301
temperature of diseased and healthy leaves measurement, 676
tests for fungicidal solution strength for bulb dips, 598
toxins demonstrated in tomato Fusarium wilt by intergeneric grafts, 470
ultra violet absorption in determining concentration of purified TMV, 488
virulence retention in lyophylized culture of *Pseudomonas caryophylli*, 406
virus assay, quantitative method for stone-fruit viruses on cucurbits, 175
virus cherry seed transmission, quick method, 467
virus concentration estimation, biological assay and ultraviolet absorption method compared, 589
virus inoculum, flower tissue, 479
virus purification, electrophoresis and density gradient centrifugation, 467
wheat breeding for rust resistance, "composite variety," 467
woody tissue, instruments for isolation of fungi, 403
Temperature, air and hot water, lethal to *Verticillium albo-atrum*, 589
air and soil, effect on turnip virus 1 strains in *Nicotiana* spp., 482, 550
air, effect on injury of olefinic peroxide fumigants on herbaceous plants, 588
bayberry-yellows virus control, 18
effect on, bacteriophage plaque formation, 28
cabbage yellows and Fusarium wilt of radish, 277
development of Fusarium wilt on flax, 492
disease development in gladiolus corms, 141
Erysiphe graminis tritici on seedlings in greenhouse, 290
gall development, corn smut, seedlings, 656
growth and disease ratings, pear seedlings in greenhouse, 160
hydrogen cyanide production by alfalfa crown rot pathogen, 581
lesion formation by potato virus Y on *Physalis floridana*, 2
longevity of spores of oak wilt pathogen, 476
longevity of *Ustilago nuda*, 407
oat varietal reaction to crown rust, 484
production and survival of sporangia of *Phytophthora infestans*, 505
radial growth of *Ustilago striiformis* varieties, 534
reaction of Kubanka wheat to races of *Puccinia graminis tritici*, 481
survival of *Colletotrichum gossypii* on cotton seeds, 220
susceptibility of barley to powdery mildew, 162
susceptibility of oat hybrids to stem rust, race 7, 472
wound periderm and cuticle, gladiolus corms, 141
heat-ultra method of purification of TMV for spectrophotometric assay, 89
hot water and air, lethal to *Verticillium albo-atrum*, 589
of diseased and healthy leaves, relation to respiration, 679
of diseased, injured and healthy leaves compared, 675
Temperature—*continued*
of host tissue as affected by parasites, 221
of *Pythium ultimum*, internal plant, related to damping-off by, 318
of root and stem, internal, compared with soil, 317
relation to internal cork development, sweet potato roots, 50
soil and air, effect on turnip virus 1 strains in *Nicotiana* spp., 482, 550
soil, effect on gladiolus reaction to Botrytis neck rot, 167
—time gradients control, mushroom-spawn nematode, 404
Terramycin, see Antibiotics
Tetrachloronitrobenzene, effect of, on Fusarium dry rot in potatoes, 95
Tetrachloro-para-benzoquinone, see Fungicides, chloranil
Tetramethylthiuram disulfide, see Fungicides, thiram
Thecaphora spp., nomenclature, changes in, 183
Thelephoraceae, wood rotting by, 21
Thielaviopsis basicola, tobacco breeding for resistance to, 239
Thiolutin, see Antibiotics
Thiouracil, tobacco mosaic virus, inhibition of, related to light, 555
THIRUMALACHAR, M. J., 527, 608, 645
THOMAS, H. EARL, 218
THOMAS, R. REX, 11, (407)
THOMAS, W. D., JR., (587)
THOMPSON, G. E., 293
THORNBERRY, H. H., 486
THURSTON, H. DAVID, 486
THURSTON, H. W., JR., (108)
TIFFANY, LOIS H., 486
Tilletia asperifolia, spore germination of, 219
brevifaciens, spore survival, exposed on soil, 487
caries, conditions affecting resistance of wheat, 473
differential survival, *T. foetida* in mixture on host, 558
hybridization, *T. foetida*, 559
pathogenic biotypes, 399
sex compatibility factors, 322
sporidial fusion in culture, 322
foetida, differential survival, *T. caries* in mixture on host, 558
hybridization, *T. caries*, 559
spp., nomenclature, changes in, 183
taxonomy, spore germination related to, 219
TIMIAN, ROLAND G., 487
TIMS, E. C., 294
TINLINE, R. D., 487
Tobacco, bacterial wilt, resistance to, 240
black root rot, resistance to, 239
black shank, field inoculation effectiveness for testing resistance, 289
root-knot nematode effect on resistance, 483
tomato fruit rot incited by, 290
toxin production, 294
blue mold control, 486, 616
distribution, geographical, 616
oospores of pathogen, germination, 701
overwintering of pathogen, 616
spore showers, 616
zoospores, infection by, 701
breeding for resistance, 239, 243, 469
copper sulphate toxicity symptoms on seedlings, 125
cyst nematode, host preference, 106
damping-off of seedlings, control of, 125
disease resistance, 239, 243, 469
etch virus, 564
Fusarium spp., 590
mosaic virus assay, see also under Viruses, 77, 89, 484, 485, 589
effect of organic compounds on multiplication, 589
effect on temperature of leaves, 679
growth curve of host in relation to concentration curve of virus, 488

Tobacco—*continued*
host nutrition in relation to virus multiplication, 488
pressure effects on, 70
radiophosphorus inhibition of, 206
resistance to, 242, 475
strains, 587
thiouracil, control and plant injury, 555
necrosis virus, 25, 590, 679
Pythium ultimum on roots, 590
radiophosphorus injury, 207
resistance to combination of diseases, 240
ring spot virus, 70, 485, 687
soil treatment effect on growth, 590
thiouracil up-take by leaves, related to light, mosaic virus studies, 556
Tomato, awl nematode, 296
bacterial canker, seed transmission, and control, 473
buckeye rot, susceptibility to, 290
bushy-stunt virus strains of, 485
damping-off seed treatment, 291
disease resistance, effect of growth-regulators, 137
early blight, control, 25, 478
Fusarium wilt, chemotherapy, 43, 137
copper ion effect on, 283
enzymes role in etiology of, 470
ethyl alcohol relation to, 663
ethylene, epinasty, 470, 663
ethylene production in infected plants, 664
fungus distribution, 116
iron ion effect on, 283
lycomarasmin inhibitors effect on, 283
lycomarasmin role in, 284
mechanism of vascular discoloration, and wilting, 470
metabolites production of vascular browning, 490
nutritive sprays effect on, 466
osmotic properties of leaf parenchyma in diseased plants, 619
pectic enzymes, 472, 535
physiology, 116
8-quinolinol effect on, 283
reduction of water flow estimated by scale model, 488
resistance affected by metabolic inhibitors, 483
toxin demonstrated by intergeneric grafts, 470
transpiration, velocity, 621
vascular reactions to, 116
golden nematode, cytological effects of, 471
internal temperatures of root and stem compared with soil, 317
iron deficiency, effect on response to lycomarasmin, 116
late blight, resistant varieties reaction to virulent race of pathogen, 344
sporangia production and survival, 505
leaf mold, varieties immune from, 109
lycomarasmin effect on, 195, 281
nutrition, effect on susceptibility to *Alternaria solani*, 476
root-knot control, 465, 485
root-knot, resistance to, 290
root rot control, 465
stubby-root nematode, 296
tobacco cyst nematode, 106
toxic metabolites, effect on, 116
Verticillium, soil fumigation tests and control, 595
virus, apparently new, 480
Toxic metabolites, lycomarasmin, 116
vascular browning production, 490
Toximycin, see Antibiotics
Toxins, abolition of regulatory mechanisms in hosts, 221
defense reaction and pathogen, chemical aspects, 353
2,4, dinitrophenol, effect on respiration compared, 224
effect on phosphorus metabolism, 225
modes of action, 355
nature of defense reaction to, 351
physiology of formation in microorganisms, 355
produced by *Fusarium*, 352
Toxins—*continued*
production by host—parasite interaction in tomato Fusarium wilt, 470
production in relation to tobacco black shank, 294
proteins, carnation wilt studies, 587
radioisotopes, use in detecting toxin and host tissue reactions, 236
respiration, effect on, 221
tobacco black shank, adsorption on charcoal, 294
tomato Fusarium wilt, effect upon species grafted to tomato stocks, 470
vivotoxins, 229
wound reactions to, 352
Toxoptera graminum, see Insects aphids, greenbug
Transpiration, magnesium and potassium salts effect on tomatoes in relation to Fusarium wilt, 466
N-trichloromethylthio tetrahydrophthalimide, see also Fungicides, captan, coined name, 58
Trichoderma spp., corn seedling blight, associated with, 477
in soil, inhibitory to damping-off of peppers, 586
Trichodorus sp., see Nematode, stubby-root
Trichothecium roseum on peanuts in market, 474
2,3,5 triiodobenzoic acid, Fusarium wilt of tomato, effect upon, 137
metabolism of host, effect upon, 139
Triticum dicoccum, wheat stem rust, breeding for resistance, 472
timopheevi, powdery mildew resistance to, 290
Tritium, see radioisotopes
Tropaeolum majus, see Nasturtium
TROXEL, A. W., (477), 590
TRUE, R. P., 487
TRYON, E. H., (487)
Tuburcinia spp., nomenclature, changes in, 183
Tumors, see Galls
Turf, see also Grasses, brown patch (*Rhizoctonia solani*) factors influencing, 484
brown patch, susceptibility of varieties to, 110
Curvularia "fading-out," 109
TURNER, W. F., (691)
Turnip, virus 1 strains, effect of soil and air temperatures on in *Nicotiana* spp., 482, 550
Tylenchorhynchus, see Nematodes
TYLER, L. J., 487
Tyloses formation in oak wilt, 451
TYNER, L. E., 313

Ultra violet, absorption method for estimation of TMV concentration, 589
spectra, tobacco mosaic virus, groups of strains compared, 587
Uredo spp., nomenclature, changes in, 183
Urocystis spp., nomenclature, changes in, 183
Uromyces betae, sugar beet resistant varieties, 300
fallens, effect on respiration of clover leaves, 223
phaseoli, pressure effects on, 70
Usnic acid, see Antibiotics
Ustilagidium spp., nomenclature, changes in, 183
Ustilago bullata, differential grasses, 201
races, new, 200
x *U. striiformis hordei*, inheritance of spore and sorus characters, 547
nuda, control on barley and wheat, seed treatments, 313
differential barley varieties, 407
longevity, 407
races, 369, 407
races, spore germination characteristics, 482
temperature effect on longevity, 407
spp., ergot associated with, 461
inoculation method, barley and oats, 697
nomenclature, changes in, 183
striiformis, effect of temperature on radial growth, varieties, 534
hordei, x *U. bullata*, inheritance of sorus and spore

Ustilago striiformis—*continued*
characters, 547
host range, 527
nomenclature, 530
varieties and races, 528
varieties, spore germination, cultural characters and cytology compared, 527
tritici, effect on respiration of wheat leaves and stems, 223
races, 681
races, spore germination characteristics, 482
wheat germ plasm effect on prevalence of races, 683
zeae, amino acids synthesized by, 470
partial vacuum method of inoculating corn seedlings, 654
pathogenicity of biotypes, method to determine, 654
spore germination, effect of potassium phosphate, 478

Vaccinium australi, see Blueberry
VALLEAU, W. D., 616
VALLEGA, JOSE, (471), 487
VAN ARSDEL, E. P., 487
VANTERPOOL, T. C., 488
VATTER, A. E., (486)
VAUGHAN, EDWARD K., (589), 590
Vaughan, Richard English, Biographical sketch, 349
Venturia inaequalis, amino acids as nitrogen sources, 481
races 2 and 3, physiologic specialization, 483
Verticillium albo-atrum, control, 376, 475, 594
eggplant, isolates, 466
Fusarium associated with, on potatoes, 108
Gossypium spp., reaction to, 489
host range, 588
internal brown spot associated with, on potatoes, 108
microflora and nematodes associated with sclerotia in soil, 594
pathogenicity of isolates on peppermint, 588
pepper, isolates, 466
"pink eye" associated with, on potatoes, 108
Pseudomonas fluorescens, associated with, 108
soil fumigation tests, methods of evaluating effects, 593
storage behavior of potatoes infected with, 108
temperatures lethal to, hot water and air, 589
Vicia americana, see Common vetch
faba, see Bean, broad
Vigna spp., see Cowpea
Vinca rosea, see Periwinkle
Viruses, general, acquisition threshold periods and vector efficiency in 4 species of aphids, 30
concentration in plant tissue, biological assay, ultraviolet absorption method compared, 589
effect on temperature of infected leaves, 679
electron microscopy of virus particles, 486
infectivity inhibited by synthetic polypeptides, 473
inoculum, flower tissue for isolations, 479
loss of insect vector transmissibility by, 466
mosaic virus complex on Ladino clover, 271
phony disease, peach and plum, transmission of virus from tops of peach and plum trees, 691
purification, electrophoresis and density gradient centrifugation, 467
quantitative method of assay of stone-fruit viruses on cucumber, 175
Viruses,
alfalfa mosaic, differentiation of strains, 479
local lesion strain, 275
strains, host range, 271
systemic strain, 275
thermal inactivation, 274
tomato varieties, 479
Aureogenus spp., insect vectors, 9
loss of insect vector transmissibility, 466
purification, 467
bayberry-yellows, 15
bean 2, gladiolus, 111

Viruses—*continued*
pea, resistant variety, New Era, 473
bean southern mosaic, serological test for, 77
bean yellow mosaic, age of plant and method of inoculation, 473
mild and necrotic strains, 275
necrotic-lesion strain, cross-protection, 45
on gladiolus, 114
on tobacco and bean varieties, 11
red clover symptoms, 470
severe-yellow-mosaic strain, 11
strains, host range, 271
Brassica nigra, age, effect on transmission of, 212
age of plant, effect on plant susceptibility, 211
aphid-host plants interaction variation, 209
aphid transmission, 542
darkness, effect on plant susceptibility, 210
host range, 541
on *B. juncea*, 209
properties: thermal inactivation, dilution end-point, longevity *in vitro*, 541
site of inoculation, effect of, on transmission by green peach aphid, 209
source, effect on transmission of, 211
cabbage A, effect of air and soil temperatures on, in *Nicotiana* spp., 550
cabbage black ring, effect of air temperature on, in *Nicotiana* spp., 550
cabbage mosaic, Badger Market, new variety, resistance to, 650
camellia, transmitted, 293
cauliflower 1 group, properties, 545
celery western mosaic, aphid transmission, 29
cereal yellow-dwarf, aphid vectors, 132, 309, 312
control, 312
epiphytotic, conditions favoring, 309, 312
grass species immune from, 310
host range, 135, 309
red-spot mosaic, compared, 135
symptomless carriers, 309
cherry complex, flower tissue inoculum, 479
cherry green ring mottle, 481
cherry necrotic ring spot, quick test for seed transmission, 467
cherry recurrent chlorotic ring pattern, 325
cherry ring spot, effect on growth of nursery trees, 480
cherry tatter leaf, infectivity and longevity, of inoculum, 326
cherry yellows, effect of aging on infectivity of inoculum from vegetable marrow and pumpkin, 325
infectivity and longevity in cucurbit hosts, 324
inhibitors of infectivity of isolates of, 330
quick test for seed transmission, 467
transmission from peach understock to Japanese plum, 108
Chlorogenus myricae, 19
chrysanthemum stunt, *Marmor chrysanthemi*, proposed name, 406
properties: dilution end-point, longevity *in vitro*, thermal inactivation, 406
citrus tristeza, 51
crucifer, aphid-transmissible, radish 1 group, 545
thermal inactivation and dilution end-point of strains, 545
cucumber mosaic, gladiolus, 111
inoculum, gladiolus flower tissue, 479
on Japanese barberry, 489
strains from alfalfa and pepper on pepper, 333
grape degeneration, related to Pierce's disease, 611
yellow-headed sharpshooter vector, 612
grape Pierce's disease, leafhopper vector, 293
grape yellow mosaic, 475
horseradish mosaic, effect of air temperature on, in *Nicotiana glutinosa*, 550
Lethum australiense, purification, 467

Viruses—*continued*

Myrica virus 1, 19
nasturtium mosaic, Brazilian compared with other nasturtium mosaics, 307
distribution of, in inoculated plants, 304
Lycopersicum virus 3 relation to, 306
properties, 307
strains of, 307
symptoms on naturally and experimentally infected plants, 304
oat Siberian mosaic and wheat striate mosaic compared, 539
panax ringspot, symptoms and transmission, 643
pea Wisconsin streak, pea aphid vector, 484
peach mosaic, spread related to age of infection, 585
peach necrotic ringspot, 108
peach western X, ninhydrin-reacting substance in leaves, 586
physiological changes induced by, 481
respiration of healthy and infected foliage, 481
peach yellow leaf roll, leafhopper transmission studies, 562
potato A, strains, 479
Myzus persicae vector, 479
potato curly-top, strain, 587
synonym green dwarf, 684
potato leaf roll, 248
on tomato, effect upon response to naphthaleneacetic acid, 109
potato mosaic, 247
potato net necrosis, 249
potato X, method for identifying immune seedlings, 487
potato Y, aphid transmission, 217
mechanical inoculation vs. aphid inoculation, 469
Physalis floridana, 1
properties and host range, Hawaii, 217
prune dwarf, infectivity and longevity, of inoculum, 326
radish 1 group, 545
red clover vein-mosaic, age of plant and method of inoculation, 473
incidence and host range, 473
pea stunt, 473
rhubarb ringspot-like, 590
rose rosette, 218
spotted wilt on tomato in Hawaii, 217
stone-fruit, in cucurbit hosts, effect of extracts on infectivity, 328
infectivity and longevity in cucurbit hosts, 324
stone-fruit, green ring mottle, 326
stone-fruit latent complex, by cherry flower inoculum, 479
stone-fruit necrotic ring-spot, 325
sugar beet curly-top, strain, nature of injury, 588
yellows relationship, 587
sugar beet yellows, 301
curly-top relationship, 587
tobacco-etch and TMV compared on pepper species, 564
Chenopodium album, new local lesion host, 568
tobacco mosaic, bean acquired resistance, 490
breaking of particles caused by freezing, 482
classification of strains, 589
effect of organic compounds on multiplication, 589
electron microscopy *in vivo*, 484
electrophoretic mobility, groups of strains compared, 587
growth curve of host in relation to concentration curve of virus, 488
host nutrition in relation to virus multiplication, 488
in tissue culture of tobacco, synthetic media, 475
isoelectric point, groups of strains compared, 587
mutants, serological tests for, 77
nitrogen, nutrition effect on concentration of, in tomato, 477
nutrition of host tissue in virus activity, 575
particles associated with granules in plant tissue revealed by electron microscopy, 484
pressure effects on, 70
purification of, by heat-ultra method for spectrophotometric assay, 89
radiophosphorus effect on, 206
rosette strain, serological test for, 77
serological test for, 77
serological tests for antigenic relationships, strains, 589
size of particles, 482
strains, continous variation, 587
strains, effect of temperature on toxicity, 587
thiouracil control and plant injury, 556
thiouracil inhibition of, related to light, 555
ultra-violet absorption spectra, groups of strains compared, 587
tobacco necrosis virus, effect on temperature of bean leaves, 679
strain, host adaptation, bean and petunia, 590
tobacco ring spot, activity and particle sedimentation and counts from electron micrographs, 688
electron microscope studies, 687
electrophoretic fractionation and mobility, 687
hexagonal particles, concentration and purification, 485
on gladiolus, identification of strains, 112
particle size and shape, 688
pressure effects on, 70
purification, 485, 687
tomato, apparently new, described, TMV compared, 480
tomato aspermy, aphid vectors, on chrysanthemum and tobacco, 404
on various plant species, 404
tomato bushy-stunt strains, 485
tomato ringspot on *Hydrangea macrophylla*, 404
turnip 1, effects of air and soil temperature on, in *Nicotiana* spp., 550
host nutrition and virus multiplication in *Nicotiana* spp., 669
properties, 545
strains, effect of soil and air temperatures on, in *Nicotiana* spp., 482
variegation, leaf and flower, camellia, graft transmitted, 293
wheat Russian mosaic and striate mosaic compared, 539
wheat streak mosaic, *Aceria tulipae* vector, 484
effect of wind and blowing soil on infection, 484
elongated particles associated with, electron microscopy, 458
wheat striate mosaic, host range, 539
leafhopper vector, 537
Russian wheat and Siberian oat mosaics compared, 539
seasonal occurrence and spread, 539
symptoms, 537
wound-tumor, filterability, 390
insect vectors, 9
pH and stability, 389
potato yellow-dwarf, stability compared, 390
properties of, 387
tomato spotted wilt, stability compared, 390
Vitamins, see also Nutrition, B_{12} absence in cedar-apple rust galls, 362
biotin effect on growth of *Chalara quercina*, 443
thiamine effect on growth of *Chalara quercina*, 443
Vitis spp., see also Grape, Melanconium leaf and stem fleck on, 347
Vivotoxins, as competitive inhibitors, 232
defined, criteria for, 229
mechanisms of action, vascular occlusion, 231
Volcani, Zafrira, 92

Wadsworth, D. F., 294, (294)
Waggoner, P. E., (195), (229), 281, 317, (319), (470), 488, (619), (663)
Waite, B. H., (700)

Walker, J. C., (116), 215, (415), (489), (490), (535), (596), 649
Wallin, J. R., 505
Walter, James M., (344)
Walters, H. J., (483)
Watermelon, anthracnose, breeding for resistance to, 254
cucumber Fusarium on, 481
Fusarium wilt, breeding for resistance to, 254
Watertor, Jean L., (475)
Watkins, G. M., (291)
Watson, R. D., 590
Weather, effect on production and survival of sporangia of *Phytophthora infestans*, 506
jelly end rot of potato effect on, 467
microclimate effect on white pine blister rust distribution, 487
Weathers, Lewis G., (482), 488, (550), (669)
Weaver, L. O., 407
Weber, George F., 488
Weed, Richard M., (480)
Weihing, John L., 488
Weinberger, J. H., (691)
Weintraub, M., (175), (324), 328
Weiss, Freeman, 407
Wheat, black stem rust, races of pathogen manner of infection of resistant varieties, 485
bunt, conditions affecting resistance, 473
control, 393
fungi, survival in mixture on host, 558
pathogenicity of physiologic races and inbred lines of pathogen, 398
cereal yellow-dwarf virus, aphid vectors, 309, 312
epiphytotic, conditions favoring, 310
host range, 309
crown root infection and rootrot, 484
crown rot, Hessian fly relation to, 467
disease distribution in Oklahoma, 294
ergot associated with loose smut, 461
foot rot, Hessian fly relation to, 467
Helminthosporium spp., reactions to isolates of, 517
Kubanka, *Puccinia graminis tritici*, effect of light and temperature on reaction to races of, 481
powdery mildew, seedling varieties in greenhouse, resistance, 290
temperature effect on, in greenhouse, 290
rootrot, 484
control, biological, new method, 701
Russian mosaic virus and striate mosaic compared, 539
rust, breeding for resistance, "composite variety," 467
rust, leaf, control by and phytotoxicity of chemotherapeutants, 496
distribution in Oklahoma, 294
races, 294
rust, stem, breeding for resistance, 472
chemotherapy, 659
control by and phytotoxicity of chemotherapeutants, 496
races, pathogenicity on Kenya varieties, 474
seedling and adult changes in resistance, 468
spore shower, origin and extent, 471
smut, covered, control, 461
smut, loose, control, seed treatment, 315
distribution in Oklahoma, 294
ergot associated with, 461
races of pathogen on varieties, 682
races, spore germination characteristics, 482
resistance of Kawvale, to, 683
Wheat—*continued*
speckled leaf blotch, distribution in Oklahoma, 294
streak mosaic virus, *Aceria tulipae* vector, 485
effect of wind and blowing soil on infection, 484
striate mosaic virus, host range, 539
leafhopper vector, 537
Russan wheat and Siberian oat mosaics compared, 539
seasonal occurrence and spread, 539
symptoms, 537
varietal reactions to diseases in Oklahoma, 294
yellow-dwarf virus, 131
effect on yield, 135
yellow rust, races, inheritance of resistance, 471
Wheeler, H. E., 236, 489
Wiant, James Stewart, Biographical sketch, 592
Wilbur, Wesley D., (469)
Wildfire toxin, 232
Wildman, S. G., (587), (589)
Wiles, A. B., (215), 489
Wilhelm, Stephen, (589), 590, 593
Wilkinson, R. E., 489
Williams, Edwin B., (483)
Williams, Lansing E., 489
Williamson, C. E., 489
Willison, R. S., 175, 324, (328)
Wilson, J. D., 489
Wilson, R. A., (405)
Winstead, N. N., 489, 490
Wolf, Frederick T., 294
Wollman, E. S. H., 490
Wood decay in buildings, 20
Wood rot, citrus, concentric canker, 99
Fomes applanatus, associated with, on citrus, 99
Woody tissue, instruments for isolation of fungi, 403
Wooley, Paul H., (589), 590
Wound cork, Fusarium dry rot of potatoes, effect of, on penetration of, 95
Wound hormones, 352
Wound periderm and cuticle, gladiolus corms, effect of heat-curing on, 141

Xanthomonas pelargonii on geranium, 588
phaseoli var. *sojensis*, field and laboratory tests on persistence, 189
pruni, bacteriophage, factors affecting plaque formation, 27
terramycin control on peach, 405
Xiphinema americanum, see Nematodes, dagger

Yale, John W., Jr., (590), 590
Yarwood, C. E., 70, 490, 590, 675
Young, Donald A., 490
Young, H. C., Jr., (294), 294

Zaumeyer, W. J., (11), 38, 45, (333), 407, (480)
Zentmyer, George A., 490
Zinc dimethyl dithiocarbamate, see Fungicides, ziram
ethylenebisdithiocarbamate, see Fungicides, zineb
Zinnia, cucumber mosaic virus strains on, cross-protection, 336
Zinnia elegans, nasturtium mosaic virus on, 306
Zscheile, F. P., (473)
Zuckerman, Bert M., (61), (65), 287, 490
Zygophiala jamaicensis, carnation greasy spot, 585
Microthyriella rubi, relation to, 470

ERRATA, VOLUME 42

Page 474, column 2, line 12, *read* 9 to 14 p.p.m. Zn., *for* 40 to 63 p.p.m. of Zn.

Page 504, column 1, line 5, *read* ramannianus *for* rammanianus

line 24, *read* Nef *for* Näf

Page 505, column 2, Literature Citation 3, *read* Nef *for* Naf

Page 541, column 1, line 8, *read* Each leaf was infested. . . *for* Each leaf was infected. . .

Page 548, column 2, line 12, *read* the disease was observed to spread *for* of the diseases spread

Page 599, column 2, line 9, *read* 6.2 *for* 62

Page 601, column 1, line 5, *read* mc. *for* cm.

Page 645, column 2, line 7 from bottom, *read* P'3 *for* P'2

Page 665, Table 2, Footnote a, *read* which survived 42 days *for* which survived 30 days

Page 668, Literature Citation 8, *read* 646-705 *for* 464-705

ERRATA, VOLUME 43

Page 30, column 1, line 12 should be line 14, *to read* The 4 species of aphids were given acquisition feeding periods of 5, 10, 15, 20, 25, and 30 seconds after a preliminary starvation period of 30-91 minutes ($\bar{x}$=75.04 min.)

Page 30, column 1, line 31, *read* lily *for* celery

Page 85, Table 4, column 4 "Average green wt./plantable seedling", *delete* reference to footnote "a".

Page 109, column 1, line 1, *read* Fusicoccum amygdali Delacroix *for* Fusicoccum persicae E. & E.

Page 129, legend for Fig. 1. A) *read* Club Mariout *for* Club Marious

Page 153, column 1, line 32, *read* (r=–0.907 *for* r=0.907)

Page 153, column 2, line 3, *read* —0.883 *for* 0.883

Page 198, column 1, line 43, *read* polysaccharide *for* polysaccharaide

Page 215, column 1, footnote 1, *read* Foster *for* Porter

read Owen *for* Omen

Page 282, legend for Fig. 1, salt concentration, *read* N *for* M

Page 291, column 1, line 32, *read* Fusarium oxysporum f. tracheiphilum (E. F. Sm.) Snyder & Hansen *for* Fusarium conglutinans Wr.

Page 319, column 2, line 4 from bottom, *read* at pH 6 than at pH 3.6 at the temperatures in Table 1 *for* at pH 6 and pH 3.6 than at the temperature of Table 1

Page 320, column T, equation 2, *read* ln *for* In

Page 321, Literature Citation 2, *read* 1953 *for* 1935

Page 324, column 1, footnote 4, *insert* 1953

Page 350, column 2, Reference 1921, *read* Fernow *for* Fernon

Page 358, column 2, line 17, *read* pH to 7.2 *for* pH 7.2

Page 361, column 1, line 2 from bottom, *read* is *for* in

Page 361, column 2, legend for Fig. 1, *read* Bison Flax *for* Bisox Flax

Page 375, Literature Citation 1, *read* 1952 *for* 1925

Page 379, column 2, legend for Fig. 1 *read* Alfalfa seeds: top, from noninfected plants; bottom, from plants infected with Ascochyta imperfecta

Page 432, column 1, line 17, *read* Halsted *for* Halstead

Page 433, column 2, Literature Citation 1, *read* Halsted *for* Halstead

Page 441, column 1, line 30, *read* tap-water and distilled water rinses *for* tap-water rinses

Page 448, column 2, line 29, *read* 2-3½-in. *for* 2-3½ in.

Page 451, column 1, line 46, *read* noninoculated *for* inoculated

Page 452, column 1, line 46, *read* refoliation *for* defoliation

Page 473, column 2, line 15, *delete* "and mycelium was detected in all plants of both varieties at all stages of growth."

Page 538, column 2, line 3, *read* mosaic-infected *for* mosaic-infested

Page 540, Literature Cited, No. 10, *read* 1940 *for* 1950

DIRECTORY:

Page D8, Councilors-at-Large, column 2, *read* 1952-53 G. A. Zentmyer *for* G. W. Fischer, and *add* 1953-54 G. W. Fischer

Page D29, column 1, *read* Matsumoto, Takashi (Emeritus) Professor *for* Matsumoto, Takashi, Emeritus Professor

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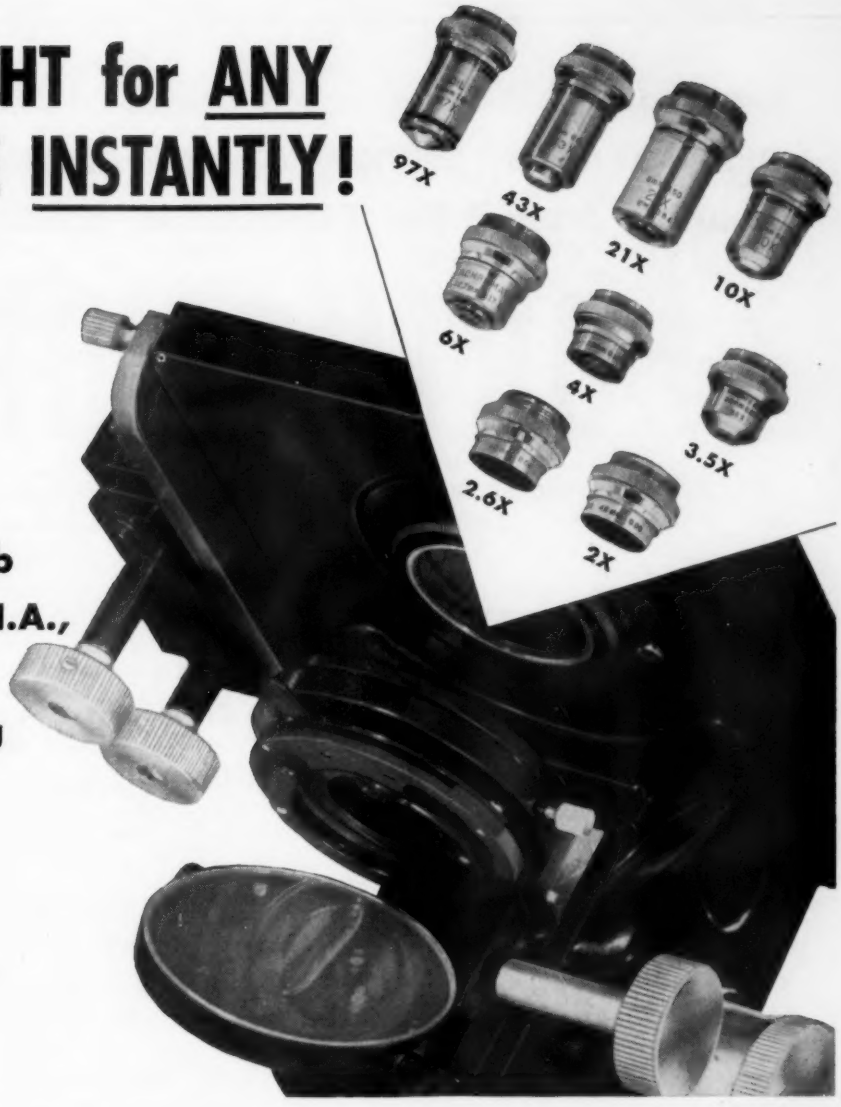

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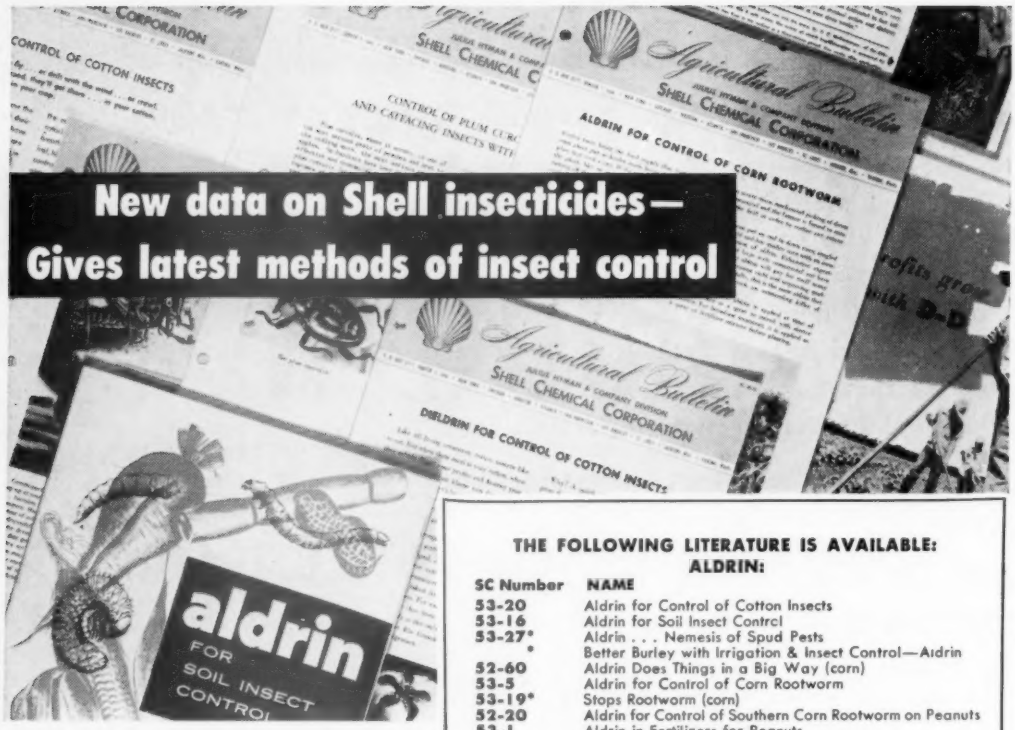

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*	Better Burley with Irrigation & Insect Control—Aldrin
52-60	Aldrin Does Things in a Big Way (corn)
53-5	Aldrin for Control of Corn Rootworm
53-19*	Stops Rootworm (corn)
52-20	Aldrin for Control of Southern Corn Rootworm on Peanuts
53-1	Aldrin in Fertilizers for Peanuts
	Control of Southern Rootworm on Peanuts (USDA Bull. EC-23)
53-15	Aldrin for Control of Grasshoppers

DIELDRIN:

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53-14*	Dieldrin Stops Boll Weevil
53-21	Dieldrin for Control of Cotton Insects in Tex. & Okla.
53-22	Dieldrin for Control of Cotton Insects
53-38	Chinch Bug Control with Dieldrin (corn)
52-56	Early Season Control of Adult Alfalfa Weevil with Dieldrin
53-47	Lawn Insect Control with Dieldrin
52-37	Dieldrin for Control of Pests Affecting Public Health
53-26*	Dieldrin, New Weapon Against Houseflies
53-11	Control of Plum Curculio & Catfacing Insects with Dieldrin
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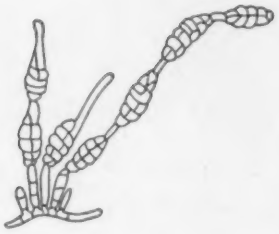

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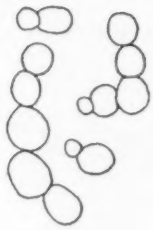

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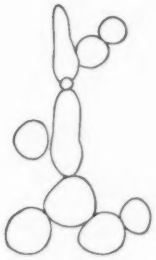

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FROM FLORIDA—excellent control of late blight on tomato seed bed plants—Phygon best of 12 chemicals tested.

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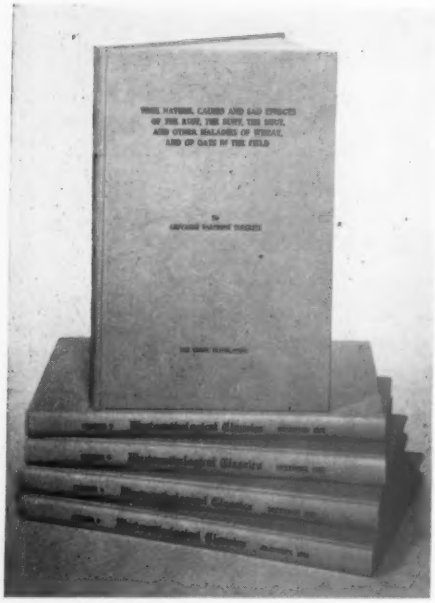

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NOTICE

Robertson copper fungicide has been thoroughly field tested and is now commercially available

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Tomatoes
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After thorough field tests, Robertson Fungicide is now commercially available. An extremely fine powder consisting of pure metallic copper, coated with regenerative cuprous oxide, Robertson Fungicide is well suited to spray-tank application; it wets easily, suspends well, passes freely through hose lines and nozzles, and disperses readily. It is not gummy, yet its adhesive quality is extremely high. Despite its low phytotoxicity, it has excellent fungicidal activity. Why not include this highly effective new fungicide in your testing program for 1954?

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The 1954 meeting will be held August 25, 26 and 27 at the Estes Park Conference Camp, Estes Park, Colorado. The camp is operated for the West Central and Southwest Areas of the Young Men's Christian Association and is widely known as the "Y.M.C.A. of the Rockies." The camp has been in operation since 1907 and comprises some 1000 acres of land. It is situated near Estes Park village, which is one of the outstanding tourist vacation spots in America. It adjoins Rocky Mountain National Park, which embraces 405 square miles of unsurpassed mountain scenery, including 65 peaks over 10,000 feet high. The camp is situated about 70 miles northwest of Denver and is accessible by car over surfaced highways. Railroad and bus tickets may be purchased direct to Estes Park. Taxi service from Estes Park village to the camp costs $1.00. The Committee on Local Arrangements suggests that members might like to make this meeting a vacation for the entire family.

The Committee on Local Arrangements for the Estes Park meeting is as follows: General Chairman, J. A. Pinckard; Vice Chairman, W. J. Henderson; Housing and Transportation, R. H. Porter, L. A. Schaal, and G. H. Lane; Registration, Norman Gerhold, John Vaughn, J. A. Pinckard and W. J. Henderson; Program, E. F. Feichtmeir, C. E. Seliskar, H. J. Walters, H. B. Hewitt; Entertainment, R. R. Baker, Norman Gerhold, William Riedel, and W. J. Henderson; Visual Aids, Ross Davidson and C. W. Frutchey.

Meeting accommodations consist of several conference halls adequate for accommodating from 150 to 600 people. In addition, there is a large rustic administration lodge.

Lodging accommodations consisting of comfortable rustic cabins and lodges will be available to members and their families. The facilities can handle 600 to 700 people. Other than cabins, there are four large rustic lodges with comfortable sleeping quarters surrounding a large lobby with fireplace and lounging facilities. A hostess will be in charge of each lodge. Maid service, all bedding and towels will be furnished. Shower baths, hot and cold running water, lavatories and toilet facilities are conveniently located to accommodate guests of cabins and lodges.

Dining Room Service. Two large rustic type dining rooms, each of which can seat 350 guests, will be available.

Rates for members and their families include meals from evening dinner, August 24, to breakfast, August 28, and lodging in cabins or lodges. The complete rate for adults is $25.00 each. Children, ages 2-6 years, $18.00 each. Children, ages 7-11 years, $21.00 each. The daily rate for adults is $6.50 each. Children, 2-6 years, $4.75 each. Children, 7-11 years, $5.50 each.

Staff of the Conference Camp consists of 150 people, many of whom are college students who serve the guests.

Phytopathologist K. Bagchee, Chief Research Officer, Mycology Branch, Forest Research Institute, Dehra Dun, India, who retired after 26 years of service, was reappointed as Mycologist in the same Institute. Dr. Bagchee conducted extensive work on inspection of timber during the last war and surveyed diseases of tropical forests in the plains. His earlier work was on biology and control of the coniferous rusts and diseases of hardwoods and conifers of India.

A book, "The Ustilaginales of the World," by the late George L. Zundel has been multilithed by the Pennsylvania Agricultural Experiment Station. This monograph represents the life work of one of the leading Ustilaginalists. It consists of descriptions, host symptoms, synonyms, type hosts, and distribution of all known smut species, together with a host index and an index of synonyms. The manuscript was completed in 1945 but publication was delayed because of World War II and other difficulties. It sells for $4.00.

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BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia 4, Pa.

TOBACCO requires too much labor and attention for the grower to risk producing an inferior crop by using inferior seed. It is hard enough to bring the crop successfully from seed bed through curing barn even under good conditions. When the seed is not well-cleaned, and is not of the best quality that the grower can obtain, the prospects for a superior crop are slim, indeed. The difference between the cost of good seed and poor seed is so small that no grower can afford to use anything but the best obtainable.

Healthy tobacco plants of high quality and yield produce the best seed.

Start with *Good Seed* for Best Tobacco Crops

Although a tobacco seed is one of the smallest of field crop seed, the plant that emerges is not only of comparatively large size but is one of the most important agricultural crops, economically. In one ounce there are approximately 350,000 seed. Embedded in the tiny germs of these small seed are the genes or hereditary factors that, together with soil and climate, govern all the qualities and characteristics of the plant—its size, general growth habits, disease resistance, yield, and quality of leaf. It is therefore highly important to choose a variety well-suited to the grower's conditions and to be sure that such seed is of high quality and from a reliable source.

A field of tobacco seed about ready for harvest.

The variety the grower chooses can have a great influence on yield and quality. Federal and State Agricultural Experiment Stations and commercial seed producers have developed varieties whose seed carry factors resistant to such diseases as Granville Wilt and Black Root Rot. Disease resistance is an important consideration where these diseases are known to be present. However, the first consideration in selecting a variety should be good leaf quality with widely accepted performance over the years.

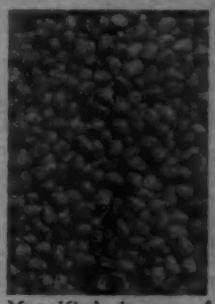
GROWING AND CARE OF SEED OF VITAL IMPORTANCE

The seedsman, in growing, processing, and distributing tobacco seed must exercise unusual care in handling tobacco seed fields and seed throughout the season. To supply the demands of growers over a wide range of soil conditions, the seedsman usually produces many varieties. It is necessary to grow these on isolated farms, or far enough apart to prevent any cross pollination. Fields must be checked and rogued during the growing season to find and remove any diseased or off-type plants. Seed must be harvested, cleaned, and packaged carefully, so that the farmer receives only high-quality seed that is uniformly representative for each variety, and that meets requirements of Crop Improvement Associations. A package of seed should be pure as to variety and free from trash, other foreign matter, and immature seed.

GOOD SEED CHEAPEST

Since the quantity of tobacco seed required to produce plants for an acre costs little more than a pound of good leaf tobacco, it seems unwise for the tobacco farmer to plant other than the best seed obtainable. Considering the high cost of producing an acre of tobacco, the farmer should take advantage of every opportunity to increase his income. An investment of only a few cents extra per acre to obtain pedigreed seed should help him realize additional dividends from his tobacco crop. Curing quality, general growth characteristics, and disease reaction of the plants that go into the planting stock which produces the seed, are important points to consider in choosing seed. It also helps if the manufacturing and smoking qualities of plant lines can be determined. It is a recognized fact concerning farm seed in general that known purity, quality, and record of performance are factors that cannot be overlooked in producing a crop economically.

The tobacco farmer should consult his County Agent, other Extension Workers, or Vocational Agriculture Teachers for help in obtaining tobacco seed of the most adaptable varieties for the conditions on his farm.



Magnified close-up of well-cleaned tobacco seed. For uniform quality, only plump and viable seed should be used. This is the type of seed the grower should obtain.

Magnified close-up of tobacco seed not thoroughly cleaned, containing foreign matter and immature seed. Growers should be on guard against seed offered in this condition.

The American Tobacco Company
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Flue-cured advertisement appearing in farm publications. It is representative of the series started in the Burley area and later extended to the Flue-cured area.

CAPTAN 50-W

(FUNGICIDE 406)

THE EXCEPTIONAL NEW ORGANIC FUNGICIDE FOR FRUITS, VEGETABLES, ORNAMENTALS

Captan 50-W has been thoroughly tested on many crops and has proven to be a superior fungicide that gives better disease control, increased yields, improved quality and longer storage life.

It is recommended for:

APPLE: Scab, Brook's Fruit Spot, Sooty Blotch, Fly Speck, Bitter Rot, Fruit Rots.
PEACH: Brown Rot and Peach Scab.
CHERRY: Leaf Spot and Brown Rot.
AVOCADO AND MANGO: Cercospora Spot or Blotch.
CUCURBITS: Angular Leaf Spot, Anthracnose and Downy Mildew.
TOMATO: Early Blight, Anthracnose, Septoria Leaf Spot, Late Blight, Stemphyllium.
POTATO: Early and Late Blight.
CARROT: Septoria and Cercospora Leaf Spot.
ROSE: Black Spot.
CELERY (Seed Bed): Damping-Off.
SEED TREATMENT: Seed Rots and Damping-Off.
AZALEA CUTTINGS: Damping-Off.

Based on good preliminary tests, Captan 50-W is recommended for trial use on:

APPLE: Black Rot (Frog-Eye).
PEARS: Pear Scab.
PLUMS: Brown Rot.
PRUNES: Brown Rot.
GRAPES: Black Rot and Mildew.
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